A STUDY ON BACTERIAL INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN DECOMPENSATED LIVER DISEASE PATIENTS IN A TERTIARY CARE HOSPITAL

Dissertation submitted to

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CERTIFICATE

This is to certify that this dissertation titled **"A STUDY ON BACTERIAL INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN DECOMPENSATED LIVER DISEASE PATIENTS IN A TERTIARY CARE HOSPITAL''** is a bonafide record of work done by **DR.J.RAJESWARI,** during the period of her Post graduate study from 2012 to 2015 under the guidance and supervision in the Institute of Microbiology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai-600003, in partial fulfillment of the requirement for **M.D. MICROBIOLOGY** Degree Examination of The Tamilnadu Dr. M.G.R. Medical University to be held in April 2015.

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DECLARATION

I declare that the dissertation titled **"A STUDY ON BACTERIAL INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN DECOMPENSATED LIVER DISEASE PATIENT IN A TERTIARY CARE HOSPITAL"** submitted by me for the degree of M.D. is the record work carried out by me during the period of **one year** under the guidance of Professor **DR.S.THASNEEM BANU, M.D.** Professor of Microbiology, Institute of Microbiology, Madras Medical College, Chennai - 3. This dissertation is submitted to the Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of degree of M.D., Microbiology (Branch IV) examinations to be held in April 2015.

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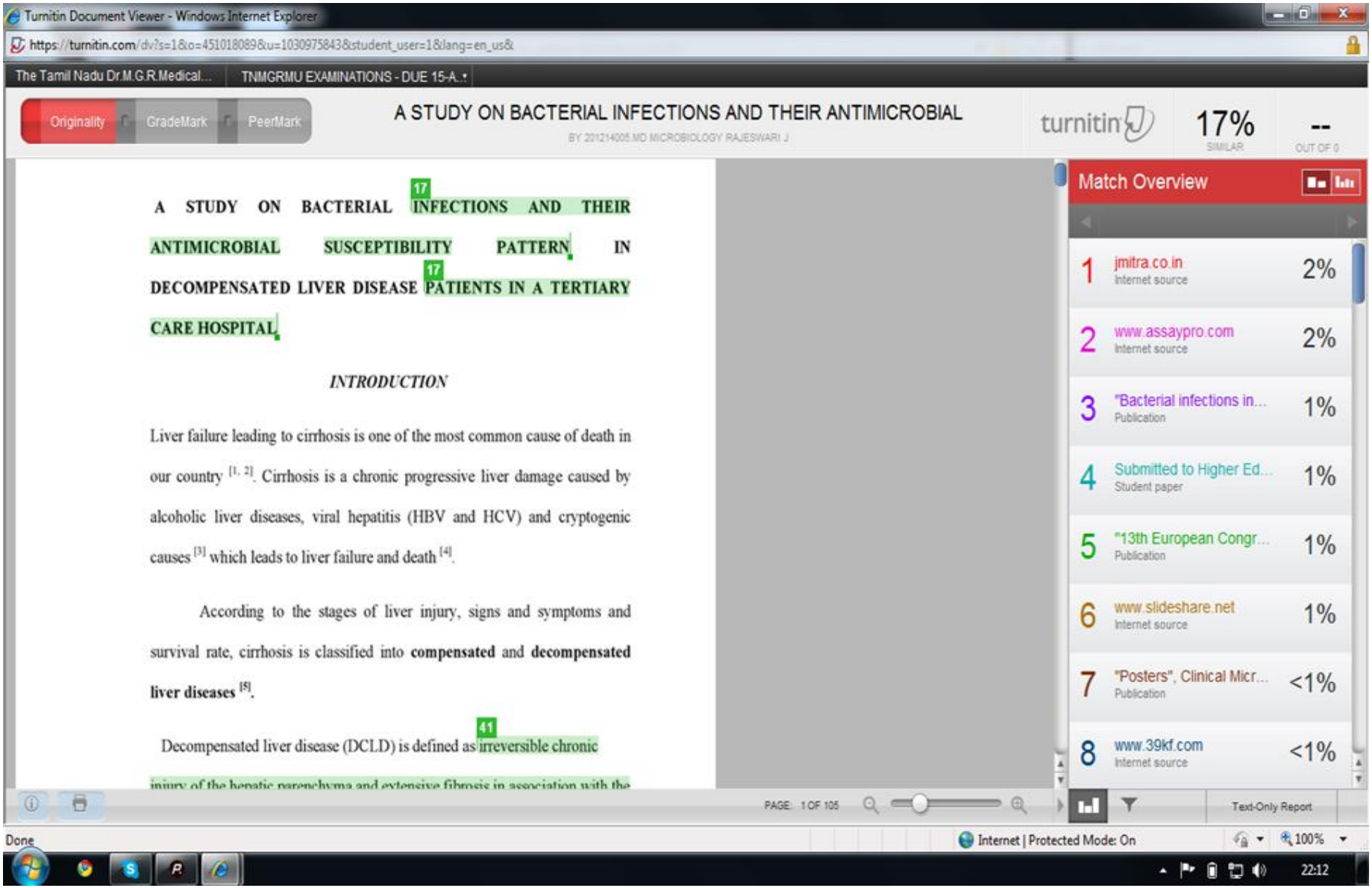
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A STUDY ON BACTERIAL INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN DECOMPENSATED LIVER DISEASE PATIENTS IN A TERTIARY CARE HOSPITAL
INTRODUCTION
Liver failure leading to cirrhosis is one of the most common cause of death in our country [1, 2]. Cirrhosis is a chronic progressive liver damage caused by alcoholic liver diseases, viral hepatitis (HBV and HCV) and cryptogenic causes [3] which leads to liver failure and death [4].
According to the stages of liver injury, signs and symptoms and survival rate, cirrhosis is classified into compensated and decompensated liver diseases [5].
Decompensated liver disease (DCLD) is defined as irreversible chronic
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A STUDY ON BACTERIAL INFECTIONS AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN IN DECOMPENSATED LIVER DISEASE PATIENTS IN A TERTIARY CARE HOSPITAL

ABSTRACT

INTRODUCTION: Bacterial infections in decompensated liver disease (DCLD) patients are one of the most frequent complications and result in high mortality and morbidity. Patients with DCLD have altered and impaired immunity, which favours bacterial infections. The most common infection in DCLD patients are spontaneous bacterial peritonitis followed by urinary tract infections, Spontaneous bacteraemia, pneumonia, and skin and soft-tissue infections. The prognosis of these patients is closely related to a prompt and accurate diagnosis and appropriate treatment decreases the mortality rates.

Aim & objective: This study was done to determine the various bacterial agents causing infections in decompensated liver disease patients and to determine the drug susceptibility and resistance pattern and to identify their associated risk factors (HBV, HCV) and to estimate the level of C3 component of complement in DCLD patients by ELISA. This study was conducted at the Institute of Microbiology, Madras Medical College, Chennai

Materials &Methods: About 150 patients (≥18 yrs), admitted in various wards of our hospital with signs & symptoms suggestive of bacterial infections in DCLD patients are included in the study. Ascitic fluid, urine, sputum and wound swab were collected. Blood and serum samples were collected from all the patients and processed according to Standard Microbiological techniques. Detection of HBsAg, Anti HCV ELISA was done according to the manufacturer guidelines to find out the associated risk factors for DCLD and to correlate the bacterial infections with complement C3 level by ELISA.

Results: A total of 150 DCLD patients, from September 2013 to August 2014, were included. The common age group involved was 41-60 years, being 97% male. The alcoholic etiology of DCLD was 84%. Out of 150 samples, culture positivity seen in 54% (81/150).In 81 culture positive isolates, 63(78%) were Gram Negative bacilli and 18 (22%) were Gram Positive cocci, which was correlated significantly [P value = 0.005]. Among 81 culture positive cases, The most common infection in DCLD patients are spontaneous bacterial peritonitis[27%] followed by urinary tract infections[26%], Spontaneous bacteraemia[19%], pneumonia[16%], and skin and soft-tissue infections[12%]. Among Gram negative bacilli,

Escherichia coli were the most common isolates and in Gram positive cocci, *Staphylococcus aureus* was the most common isolates.

Most of the organisms were 75% sensitive to amino glycosides and 50% sensitive to fluoroquinolones. All the GNB were 100% sensitive to carbapenem except one carbapenem resistant isolates, *Klebsiella oxytoca* was isolated from sputum sample.

In 81 culture-positive infections, 33[41%] drug resistant bacterial infections were identified: 27 ESBL (81%), 4 Methicillin resistant Staphylococcus aureus (10%) 1 VRE (2.3%), and 1 MBL(2.3%). Of the culture-positive infections, these drug resistant bacterial infections occurred in 11 of 21 (52%) of the UTIs, 8 of 22 (36%) of the SBP, 3 of 15 (20%) of the spontaneous bacteraemia cases, 7 of 13 (54%) of the pneumonia and 4 of 10 (40%) of the skin and soft tissue infection cases.

In our study, the prevalence of Hepatitis B surface antigen and Anti hepatitis C virus by serological methods (ELISA) were found to be 8% and 7.3% respectively.

Out of 150 total samples, Complement component C3 ELISA was done for randomly selected 88 samples with one kit due to economic constrains. Out of 88 patients, 59(67%) patients with low complement component C3 level. Of which 35(59%) patients were culture positive and 24(41%) were culture negative. 29(33%) with normal complement component C3 level, of which 25(86%) were culture negative and 4(14%) were culture positive.

Discussion: In our study, males were commonly involved in DCLD patients due to presence of risk factors like alcoholism. The main causes of DCLD were alcoholic liver diseases. The commonest age group which showed most of the bacterial infections was between 41-50 age groups. The most common and serious bacterial infections in DCLD patients were Spontaneous bacterial peritonitis than other infections. Gram negative bacilli were common isolates than Gram positive cocci. In Gram negative bacilli, *E.coli* was the main pathogen. High rate of antibiotic resistant isolates were seen in culture-positive infections, with 41% (33 of 81cases). Bacterial infections were associated with low complement component C3 level in DCLD patients.

Conclusion: The prognosis of these patients is closely related to identify the definitive etiologic diagnosis with its antimicrobial susceptibility and resistant pattern. Antibiotic prophylaxis must be restricted to selected patients and encouraging the use of first line antibiotics and to avoid unnecessary use of higher antibiotics like third generation cephalosporins will help to reduces the occurrence of new resistant strains, which can be significantly reduce hospital stay and morbidity and improve survival rate.

INTRODUCTION

Liver failure leading to cirrhosis is one of the most common causes of death in our country [1, 2]. Cirrhosis is a chronic progressive liver disorder caused by alcoholic liver diseases, viral hepatitis (HBV and HCV) and cryptogenic causes [3] which can leads to liver failure and death [4].

According to the stages of liver injury, signs and symptoms and survival rate, cirrhosis is classified into **Compensated** and **Decompensated liver diseases** [5].

Decompensated liver disease (DCLD) is defined as irreversible chronic injury of the hepatic parenchyma and extensive fibrosis in association with the formation of regenerative nodules and leading to loss of liver function [6]. DCLD is associated with one or more of the complications like ascites, portal hypertension, gastro esophageal hemorrhage and hepatic encephalopathy

Bacterial infections are more common in decompensated liver disease patients and causes 30%-50% of deaths in these patients. [7]. Spontaneous bacterial peritonitis, the (SBP) is a serious bacterial infection in decompensated liver disease patients followed by urinary tract infections (UTI), spontaneous bacteraemia, pneumonia, and skin infections [8]. The common causative organisms for bacterial infections in DCLD patients are Enterobacteriaceae, nonfermentable gram-negative bacilli and Gram positive cocci and most of them are multidrug resistant [9-10].

In DCLD patients with bacterial infections, 40% are nosocomial and\ 60% are community acquired infections [13]. Even though the incidence of bacterial infections in cirrhosis is not high but the mortality rate is very high. Bacterial infections in decompensated liver disease are due to invasive practical procedures, malnutrition [15], derangement of gut flora – intestinal stasis, bacterial over growth, increased intestinal permeability, impaired host defence mechanisms against infection.

Of the host defence mechanisms, impaired function of the reticuloendothelial system [16], deficiency of complement component level mainly C3 because C3 is synthesized by hepatocytes of liver and impaired opsonisation activity increases the susceptibility of infections in DCLD patients. Low concentrations of C3 in serum as well as low concentrations of C3 in ascetic fluid predispose to spontaneous bacterial peritonitis [17].

This study was done to determine the various bacterial agents causing infections in decompensated liver disease patients and to identify their associated risk factors and to determine the drug susceptibility and resistance pattern. Early identification of the source of bacterial infections in decompensated liver disease patients and appropriate antibiotic treatment will reduce morbidity and mortality.

AIM AND OBJECTIVES

1. To study the etiological agents causing bacterial infection in DCLD patients.

2. To determine the antimicrobial susceptibility pattern of the isolated pathogens by Disc diffusion method and Minimum inhibitory concentration by Broth dilution method.

3. 3. To find out the incidence of HBsAg and Anti HCV positivity in DCLD patients by Enzyme linked immuno sorbent assay.

4. To estimate the level of C3 component of complement in DCLD patients by Enzyme linked immunosorbent assay.

REVIEW OF LITERATURE

The name "Cirrhosis" was given by Laennec in 1826 [19], derived from the Greek word "kirrhos" means yellowish tan colour [20]. The first theory for pathogenesis of cirrhosis was advanced by Roessle in 1930.

Liver is the largest gland in the body and liver tissue contains two main cell types: Kupffer cells and Hepatocytes.[147]

Kupffer cells are a type of macrophage that capture and break down old, worn out red blood cells passing through the sinusoids.

Hepatocytes are cuboidal epithelial cells that line the sinusoids and make up the majority of cells in the liver. Hepatocytes perform most of the liver's functions – metabolism, storage, digestion, and bile production [21]. The liver has many complex functions.

NORMAL MICROFLORA OF VARIOUS PARTS OF GASTRO INTESTINAL TRACT (GIT) [118]:

Normally, the stomach contains 10^3 colony forming units (CFU) of microorganisms / ml [42]. Mainly it contains facultative Gram positive salivary microorganisms such as *Lactobacilli, aerobic Streptococci* and *Candida* species. The organisms will be numerous in achlorhydria state of the stomach [e.g.: proton pump inhibitor users] or presence of blood in the stomach.

The flora from mouth to second part of the duodenum is very scanty mainly consists of salivary microorganisms. The organisms of the normal flora will be high during achlorhydria or intestinal obstruction. The distal part of ileum and the large intestine normally contains *Escherichia coli*, *Enterococci* and obligate anaerobes such as *Bacterioides fragilis* and *Bifidobacterium* species. The number of anaerobic organisms will be 10^3 to 10^4 times higher than *Escherichia coli* [28]. Other colonic bacteria are *Streptococcus viridans*, other *anaerobic Streptococci*, *Clostridium perfringens* and *Enterobacter species*. This flora will be stable and may be altered by antibiotic therapy. Stability of normal flora is maintained by number of factors such as gut motility, local pH, mucosal binding sites and production of antibacterial substances from luminal organisms [37].

During perforation of intestine, initially a total of > 10^{11} organisms enter into peritoneal cavity. Among which only 3 to 4 types of organisms only are responsible for peritoneal infection. *Bacterioides fragilis* is the obligate anaerobe most commonly isolated after colonic perforation.

Defence mechanism

The liver functions as an organ of the immune system through the function of the Kupffer cells that line the sinusoids.

- Kupffer cells are a type of fixed macrophage that form part of the mononuclear phagocyte system along with macrophages in the spleen and lymph nodes.

- Kupffer cells play an important role by capturing and digesting bacteria, fungi, parasites, worn-out blood cells, and cellular debris.

- Kupffer cells secrete many complements.

- The large volume of blood passing through the hepatic portal system and the liver allows Kupffer cells to clean large volumes of blood very quickly.

Cirrhosis of the liver

The World Health Organization (WHO) definition for Cirrhosis is defined as: "irreversible diffuse process characterized by fibrosis and the conversion of normal liver architectures into structurally abnormal nodules" [22] and leading to loss of liver function [24].

Epidemiology:

Cirrhosis is the tenth leading cause of death for men and the twelfth for women in the United States in 2001, killing about 27,000 people each year [23]. The estimated prevalence of cirrhosis around the world is 100per 100 000 subjects. Many patient die due to cirrhosis in the fifth or sixth decade of the life. In 2002, according to the 2003 World Health Organization report, 783000 individuals died from cirrhosis. In the USA in 1998 a prevalence of more than 5.5 million cases of Chronic liver disease or cirrhosis was estimated, with a rate of 2030 cases per 100000 populations. The mortality rate was approximately 25000 deaths. The 10-year survival rate for compensated liver diseases is

nearly 90%, while the median survival after decompensation is about 2 years [24]

Patho physiology of cirrhosis:[2]

Pathogenesis of cirrhosis is complex. Death of hepatocytes, extracellular matrix deposition, and vascular reorganization are the central pathogenic processes in cirrhosis. The liver cells are injured by a chronic disease process, which then undergo inflammatory changes leading to cell death (necrosis) and fibrosis.

Etiology for cirrhosis:

The common causes of Cirrhosis in India are

Alcoholic liver diseases	-	60 to 70%
Viral hepatitis	-	10%
Cryptogenic diseases	-	10 to 15%
Biliary diseases	-	5 to 10%
Primary Hemochromotosis	-	5%
Wilson’s disease	-	Rare
Alpha 1 antitrypsin deficiency	-	Rare

Alcoholic liver disease :

Alcohol related liver disease is very common global problem. World Health Organization (WHO) estimates that 140 million people worldwide suffer from alcohol dependency, causing liver damage. Classification of liver damage due to alcohol consumption

- Alcohol fatty change (steatosis) - It may be reversible if the patients stop the consumption of alcohol.
- Acute alcoholic hepatitis- Due to consumption of large amount of alcohol for a long period. It may cause abnormal liver functions without any symptoms of liver failure.
- Cirrhosis- Alcohol causes significant liver damage; normal liver cells are replaced by fibrosis and nodules.
- End- stage Alcoholic liver disease- Death occurs due to profuse gastrointestinal bleeding, hepatic encephalopathy, overwhelming bacterial infections, renal failure and hepatocellular carcinoma.

Japan and India were previously of low prevalence, but now alcoholic cirrhosis is gradually on the rise in India [25] .

Acetaldehyde, metabolic end product of Alcohol, is a reactive molecule and it may interact with proteins and membrane lipids, causing alterations in their structure and function, which may lead to cell injury and cell death [2].

Concomitant alcohol abuse and hepatitis C virus occur in about 14% of individuals with chronic liver disease. Alcohol and HCV act simultaneously to increases the incidence of cirrhosis [26, 27].At least 80% of heavy alcohol drinkers developed steatosis, 10% - 35% develop alcoholic hepatitis and 10% will develop cirrhosis [28].

Cirrhosis due to Viral hepatitis

Cirrhosis mainly caused by Hepatitis B and Hepatitis C viruses. Hepatitis B is a major health problem in India. India is at the intermediate endemic level of hepatitis B carrier, based on the hepatitis B surface antigen (HBsAg). In India over 40 million (4 crore) populations are HBsAg carriers. These chronically infected people are at high risk of death from cirrhosis [29]. Worldwide, about 170 million individuals are infected with HCV, of which [30]80% develop chronic hepatitis C, and of those 20- 30% will develop cirrhosis over 20- 30years [3].

Cryptogenic cirrhosis:

Cryptogenic cirrhosis is defined as any cirrhosis for which the etiology is unknown

Classification of cirrhosis of liver

<u>According to the size of the nodules:</u>

1. Micro nodular cirrhosis- Nodules less than 3mm in size, uniform and encompass one lobule.

2. Macro nodular cirrhosis- Nodules more than 3mm, vary in size and encompass more than one lobule.

3. Mixed [31, 32].

According to the histological features: Portal, Post-necrotic, Post Hepatitis, Biliary, and Congestive.

According to the etiologic agents: Genetic, Toxic, Infectious, Biliary, Vascular, Cryptogenic [32].

According to the clinical presentation:

- Compensated liver diseases.
- Decompensated liver diseases.

About one third of cirrhosis are compensated type and do not produce any clinical symptoms and may be discovered at a routine examination or biochemical screen or at operation theatre for some other condition[33] but the liver still has the ability to function normally or compensate for the damage [24].

Decompensated liver diseases are an irreversible alteration of the liver architecture, consisting of hepatic fibrosis and areas of nodular regeneration [31].

Clinical features of Cirrhosis:

The following signs and symptoms may occur in the cirrhosis or due to the complication of cirrhosis.

- Jaundice
- Splenomegaly
- Visible distended veins over the abdomen
- Caput medusae
- Gynaecomastia
- Hypogonadism
- Spider angiomatis or Spider navi [34]
- Clubbing
- Dupuytren's contracture
- Fetor hepaticus
- Pedal edema due to hypo albuminaemia
- Other symptoms are weakness, fatigue, anorexia, weight loss [35].

Complication of the Decompensated liver diseases: Decompensated liver diseases are complicated by the following features

- Ascites
- Portal hypertension
- Gastroesophageal haemorrhage:
- Hepatic encephalopathy
- Malnutrition
- Abnormalities in coagulation.
- Bone disease
- Hematologic abnormality

Bacterial infections in Decompensated liver disease:

Bacterial infection is a severe complication of decompensated cirrhosis which is accounts for both longer hospital stay and increased mortality [18]. Puneeta Tandon et al [36] and Foreman MG et al [37] reported that once bacterial infections occur, it may lead to sepsis and death in DCLD patients.

Bacterial infections is usually asymptomatic in patients with decompensated liver disease, Clinical suspicion of infection must be high as the only indication may be a general deterioration in the patient's clinical state, increasing encephalopathy or renal impairment [38, 39].

Borzio M et al reported in 2001 that the bacterial infections occur in 32 to 34% of hospitalized patients with cirrhosis [18, 40] of which 45% were admitted with gastrointestinal hemorrhage [41], and is responsible for 30%-50% of deaths [7] Mathurin S et al reported in 2009 that the mean age was 51.8 (+/-8) years, and 84.8% were male. The alcoholic etiology of cirrhosis was 95.4% [13].

The most common infections in decompensated liver disease patients are spontaneous bacterial peritonitis (32.7%) [42], followed by urinary tract infection (31.8%) and pneumonia (15.9%) [43, 44]. The most frequent causative organisms are Gram-negative bacilli, mainly *Escherichia coli* (60%).

Spontaneous bacterial peritonitis or primary bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is defined as an abrupt onset of acute bacterial peritonitis without an apparent intra-abdominal source of

infection in patients with ascites and decompensated liver disease [45]. This is a frequent and severe complication of cirrhosis, with an incidence in hospitalized patients with cirrhosis of 7%-25%. Decompensated liver disease patients with low ascitic fluid (AF) protein, elevated serum bilirubin levels, low platelets and low complement component C3 level in serum and ascetic fluid, diagnostic and therapeutic paracentesis are the important risk factors for developing spontaneous bacterial peritonitis (SBP) [47, 48].

The vast majority of Spontaneous bacterial peritonitis (SBP) patients are presented with abdominal pain and fever followed by vomiting, hepatic encephalopathy, gastrointestinal bleeding.

The most frequently identified organisms in patients with Spontaneous bacterial peritonitis (SBP) are Gram-negative bacilli mainly *Escherichia coli* and Gram-positive cocci mainly *Enterococci.*

Clinical features and presenting symptoms for patients with Cirrhotic abdomen and tuberculous abdomen are very much similar, but the characteristics of the ascitic fluid are different, which are enumerated in the following [52]

Differentiation of tuberculous peritonitis from spontaneous bacterial peritonitis: [53]

Characteristics	Ascitic fluid from Cirrhotic peritonitis Specific gravity SG<1016	Ascitic fluid from Tubercular Peritonitis SG>1016
Protein	<2.5g/dl	>2.5g/dl
SAAG	>1.1	<1.1
Leukocyte count	TLC < 500, [normal] Predominantly mesothelial cells.	TLC >500 predominantly lymphocytes
ADA	<40	>40
PCR for Mycobacterium tuberculosis	Negative	Positive

SAAG - Serum-Ascites Albumin Gradient, **ADA -** Adenosine Deaminase.

TLC- Total Leukocyte Count

Urinary tract infections: Urinary tract infections (UTI) in decompensated liver disease patients are the second most common infection

next to spontaneous bacterial peritonitis. These patients are frequently presented with asymptomatic bacteriuria [55, 56]. The incidence of urinary tract infections is higher in DCLD patients, due to immunocompromised state and indwelling urinary catheters. The most frequent bacteria isolated from urinary tract infections are *Escherichia coli* and *Klebsiella pneumoniae*.

Pneumonia: Pneumonia is the third most common infection in decompensated liver disease patients, after spontaneous bacterial peritonitis and urinary tract infections, especially those with alcoholism, hepatic encephalopathy, gross ascites and management of oesophageal varices with balloon tamponade are predisposing factors. [57]. The commonly isolated organisms are Gram negative bacilli (*K. pneumonia*), *Streptococcus pneumoniae,* Haemophilus influenzae and Mycoplasma pneumonia [58, 59].

Skin and soft-tissue infections: Edema of the lower extremities and anterior abdominal wall which can be due to reduced albumin level and gross ascites are predisposed to skin infections in decompensated liver disease patients. The most common organisms causing skin infections are *Enterobacteriaceae, Staphylococcus aureus* and *Streptococcus pyogenes*, followed by *anaerobes* [60-62].

Spontaneous bacteraemia: The Porto systemic shunt circulation in DCLD patients will favour the organisms to escape from phagocytosis by hepatic reticuloendothelial system, there by establishing systemic bacteraemia. Prognosis very poor in patients with bacteraemia and decompensated liver

diseases.[49, 54]. This blood stream infection is caused by Gram negative bacteria followed by Gram positive cocci. *Escherichia coli* are the frequently isolated organism followed by *P*seudomonas *aeruginosa* [63] *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii* and *Enterobacter aerogenes*.

Risk factors for Bacterial infection in Decompensated liver disease:

- Invasive practical procedures.
- Malnutrition.
- Derangement of gut flora – intestinal stasis, bacterial over growth.
- Increased intestinal permeability.
- Impaired host defence mechanisms against infection.

Invasive practical procedures:

Insertions of intravenous or urethral catheter, diagnostic and therapeutic paracentesis, Endoscopy are highly predisposed to development of bacterial infections in decompensated cirrhotic patients. Of which 4 to 20% of bacteraemia may be caused by Intravenous catheter [64. Patients who require esophageal tamponade for bleeding varices are prone to develop aspiration pneumonia [65].

Malnutrition

Liver is mainly involved in energy and protein metabolism in our body. There are many factors involved in malnutrition of cirrhosis, including poor dietary intake, alterations in gut nutrient absorption, and alterations in protein metabolism.

Derangement of gut flora

The bacteria that are responsible for the infections are derived from the normal flora of the gastrointestinal tract [GIT] [(66)]. Increased intestinal bacterial over growth is due to altered intestinal motility and prolonged intestinal transit [67].

Decreased intestinal IgA or bile salts can favour intestinal bacterial over growth in cirrhosis which is the main mechanism for bacterial translocation [(68, 69).]

Translocation of bacteria from the gut to extra intestinal sites is one of the mechanisms for bacterial infections in DCLD patients. Bacterial translocation of a specific organism is always associated with intestinal bacterial over growth of the same organism. Gram negative enteric bacteria translocation more frequently than Gram positive organisms [70].

Increased intestinal permeability:[71]

The alterations in the structures of gastrointestinal tract by congestion of vascular system, oedema of the intestine, intracellular space widened and inflammation of the peritoneum in DCLD patients may predispose to an increase in intestinal permeability.

Impaired host defence mechanism

Immune dysfunction in the cirrhotic patients is multi factorial [72, 73]. Impaired function of the reticuloendothelial system, deficiency of complement component level mainly C3, impaired opsonisation activity also decrease in bactericidal activity [74, 75 & 76] have been implicated in the pathogenesis of the increased susceptibility to infections of patients with cirrhosis.

The phagocytic function of the reticuloendothelial cells are reduced due to intra hepatic shunting of blood in cirrhotic patients [77, 78]. The reduced serum concentration of complement and fibronectin play an important role in the decreased action of reticuloendothelial system (RES) [79].

Kupffer cell is the main component of monocyte macrophage system. Impaired Kupffer cell function in cirrhosis liver leads to significantly reduced phagocytic activity and bactericidal activity [60].

Acquired deficiency of certain complement components especially C3 in serum occur because Complement component C3 is mainly synthesized from hepatocytes of liver and its concentration in ascitic fluid is significantly

reduced in patients with advanced cirrhosis [80]. Complement component 3 is one of the important prognostic factors to assess the severity of cirrhosis [17].

The following mechanisms contribute to low level of serum complement in DCLD patients

1. Some complement component are directly synthesized by hepatic parenchymal cells and their synthesis may be reduced as a direct consequence of injury and death of hepatic parenchymal cells [81]

2. Extra hepatic synthesis of other complement components are also reduced due to metabolic disturbance associated with liver failure [82].

3. There may be circulating in activators for complement components are also present.

4. There may be increased consumption of complement by antigen-antibody complex [83].

5. Increased catabolism or increased loss of complement component into the urinary or gastrointestinal tract.

The concentration of the third component of complement (C3) in ascitic fluid and serum appears to have the best predictive value for bacterial infections [84].

MULTIRESISTANT BACTERIAL INFECTIONS IN DCLD

There is a increased prevalence of bacterial infections in DCLD patients, due to multiresistant bacteria (pathogens resistant to the main antibiotics, including β-lactams). The most common are extended-spectrum β-lactamase–producing Enterobacteriaceae, nonfermentable gram-negative bacilli (e.g., Pseudomonas aeruginosa), methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-susceptible Enterococci (VSE), and vancomycin-resistant Enterococcus (VRE). Epidemiological patterns of multi resistance are different among geographical areas. Multi resistant bacteria are more frequently isolated in nosocomial infections (35%-39%) compared with HCA (14%-20%) or community-acquired episodes (0%-4%). Type of multi resistant bacteria also varies among infections. Risk factors for multi resistant bacterial infection include current or recent hospitalization, health care support, and previous exposition to β-lactams or fluoroquinolones, including long-term Norfloxacin prophylaxis.

LABORATORY DIAGNOSIS OF BACTERIAL INFECTIONS IN DCLD

Early diagnosis and treatment of bacterial infection is pivotal in the management of patients with decompensated cirrhosis and is based on history, clinical examination and laboratory diagnosis.

Basic investigations:

1. Complete haemogram.

2. Biochemical test - Blood sugar,

   Blood urea and Serum creatinine

3. Liver function test – Total bilirubin, Direct Bilirubin

   Total protein, Albumin and Globulin

   AST, ALT

   Serum alkaline phosphatase

   Gamma glutamyl transferase

4. Renal function test

5. Serum iron and hepatic iron to rule out Hemochromotosis.

6. Anti smooth muscle antibody and anti LKM antibody- to rule out Auto immune hepatitis.

7. Anti mitochondrial antibody to rule out Primary biliary cirrhosis.

8. KF ring by slit lamp examination, serum and urinary content of copper to rule out Wilson's disease.

9. Serum α feto protein to rule out malignancy.

10. Ascitic fluid paracentasis: Cell count and biochemical test. If ascetic fluid neutrophil count more 500cell/µl diagnosis of spontaneous bacterial peritonitis.

11. Leukocyte Esterase Reagent Strip used for spot diagnosis of spontaneous bacterial peritonitis

Other investigations:

- ❖ Ultrasono gram
- ❖ Computed tomogram
- ❖ Liver biopsy – To rule out liver cell size and presence of nodule with fibrosis septa
- ❖ Radio- isotope scan – To rule out the stages of cirrhosis.

Serological investigations

- ➢ Antigen and antibody detection for hepatitis B virus
- ➢ Antibody detection for hepatitis C virus
- ➢ Detection of complement component level

Bacteriological investigations

1. Ascetic fluid culture and antibiotic sensitivity
2. Blood culture and antibiotic sensitivity

3. Sputum culture and antibiotic sensitivity

4. Urine culture and antibiotic sensitivity

5. Wound swab culture and antibiotic sensitivity.

Specimens include ascitic fluid, blood, sputum, urine and wound swab. Direct gram stain followed by culture and the organisms are identified by standard microbiological techniques and antibiotic sensitivity as per CLSI guidelines.

DETECTION OF RESISTANCE IN GRAM NEGATIVE BACILLI:

A) Extended spectrum beta lactamase (ESBL) [85,86]:

These are Bush class A plasmid mediated beta lactamase capable of hydrolyzing Penicillin and monobactams and inhibited by beta lactamase inhibitors but have no detectable activity against cephamycins or carbapenems and is produced mainly by members of family Enterobacteriaceae, and also by some non fermentors. They also carry resistance for other group of antibiotics (like amino glycosides, fluoroquinolones, cotrimoxazole etc) which narrow down the choices of antibiotics available for treatment.

Detection methods for Extended Spectrum Beta Lactamase :[87]

1. **Screening methods:** with cefotxime / Ceftriaxone / cefpodoxime / ceftazidime /aztreonam discs by disc diffusion method

2. CLSI phenotypic confirmatory methods: broth micro dilution method/disc diffusion method.

3. Other methods: Inhibitor potentiated disc diffusion test, double disc diffusion Synergy test, ESBL Epsilometer test, automated methods.

4. Molecular methods: PCR, DNA probes, PCR-RFLP, PCR-SSCP, Oligonucleotide sequencing.

b) AMPC PRODUCTION IN GRAM NEGATIVE BACILLI:

Amp C beta lactamase are Bush class C beta lactamase (plasmid or chromosomal mediated), which are resistant to all beta lactamase and also to beta lactamase inhibitor combinations. They are sensitive to 4th generation cephalosporin and to carbapenems. The main Amp C producing microbes were *Acinetobacter species* and *Klebsiella species*.

Detection methods AmpC beta lactamase :[88,89]

1. Screening methods: cefoxitin disc by disc diffusion method, Cefoxitin agar method, inhibitor based methods, AmpC disc test, modified three dimensional test, Amp C beta lactamase Epsilometer test.

2. Molecular methods: PCR based methods

C) Metallo beta lactamase in gram negative bacilli :[90, 91]

These are Bush class C beta lactamase capable of hydrolyzing carbapenems, other beta lactams and beta lactamase inhibitors with the exception of aztreonam. They are predominantly found in *Acinetobacter baumanii* and *Pseudomonas aeruginosa.*

Detection methods for MBL :

1. Screening methods: carbapenems disc (imipenam, meropenam, ertapenam etc) diffusion method.

2. Confirmatory methods: Imipenam –EDTA combined disc method, Imipenam EDTA double disc synergy test (DDST),EDTA disc potentiation test, HODGE test, MBL Epsilometer test

3. Molecular methods: PCR techniques

ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCUS AUREUS:

Penicillin resistance: [92]

Penicillin resistance has been increasingly recognized since 1945.Nearly 80% or more strains of *Staphylococcus aureus* are resistant to penicillin. It is of 3 types

Plasmid mediated resistance: It is due to the production of the enzyme penicillinase (beta lactamase mediated by plasmids. The enzyme inactivates penicillin by splitting the beta lactam rings. *Staphylococcus aureus* produce 4 types of penicillinase (A, B, C, D).These plasmids are transmitted to *Staphylococci* by transduction and conjugation. The plasmid also carry resistance to other antibiotics like erythromycin and fusidic acid.

2. Chromosomal mediated resistance:

Reduction in the affinity of penicillin binding protein on the cell wall also plays a role in mediating resistance to penicillin and other beta lactam antibiotics.

3. Tolerance to penicillin:

Staphylococci developing tolerance to penicillin are only inhibited but not killed.

METHICILLIN RESISTANCE:

Methicillin resistance *Staphylococcus aureus* (MRSA) are resistant to penicillin and beta lactam antibiotics. Resistant to Methicillin indicates resistance to all cephalosporins. Many MRSA isolates are resistant to other antimicrobial families, including amino glycosides, quinolones and macrolides.

The prevalence of MRSA has shown an increasing trend in India.In 1996, Pulimood from Vellore reported 24%[124].The following year Udaya Shankar from Pondicherry reported 20%.In 2006 Rajaduraipandi reported 37.9% from Coimbatore.[94-96]A study conducted by INSAR group ,showed that the prevalence of MRSA in our country is about 40 %.[97]

Mechanism of resistance

Mediated by mecA gene which encodes for penicillin binding protein2a (PBP2a) that has low affinity for beta lactams. mecA is carried on a mobile genetic element the Staphylococcal cassette chromosome (SCCmec). Five types of SCCmec have been reported.

- Type I, II, III- **HA-MRSA**
- Type IV a-d and V, Panton Valentine leukocidin (PVL)- with sub units lukS-PV and lukF PV-**CA-MRSA**

Other mechanisms of methicillin resistance:-

Some strains of *Staphylococcus aureus* are not intrinsically resistant to methicillin and lack mecA and PBP2a.

BORSA (Border line Resistant Staphylococcus aureus) are less susceptible to methicillin because of hyper production of normal penicillinase.

MODSA (Methicillin Intermediate *Staphylococcus aureus* show methicillin resistance due to their mechanisms and have normal PBP. Both these groups are genetically distinct from MRSA and of unknown clinical and epidemiological importance though their infections can be effectively treated with beta lactamase resistant penicillin and cephalosporins.

Detection and identification of MRSA:

MRSA can be detected by both phenotypic and genotypic methods; The ideal method for identification is by detection of mecA gene or its product PBP2a. But because of the high cost and requirement of expertise it is not performed in most clinical laboratories and phenotypic identification of intrinsic methicillin resistance is the standard method followed. A strain of Staphylococcus aureus is considered resistant to methicillin if the minimum inhibitory concentration (MIC) of oxacillin is≥4µg/ml [139]. Oxacillin is preferred as it is more stable than methicillin.

Methods of identification of MRSA:[98]

1. Screening methods: with cefoxitin/oxacillin disc by disc diffusion method

2. Confirmatory methods: Oxacillin MIC detection (by broth dilution, agar dilution, E test method), Oxacillin screen agar.

3. Molecular methods: detection of mecA gene or PBP2aprotein (its protein product)

Other methods are[99,100]

- ❖ MRSA screen Latex tests,
- ❖ Evigene MRSA kit Chromogenic agar
- ❖ MRSA Select (Bipo-rad)
- ❖ Chrome Agar MRSA (Bio connections).

Typing methods for MRSA:

1.Biotyping:

It is a method to characterize MRSA based on biochemical and morphological properties.[101].Based on the following 4 properties

- **Tween 80 hydrolysis**
- **Pigment production on Tween 80 agar**

- **Urease production**

- **Gentamicin resistance**

Based on the result MRSA isolates have been divided into 4 groups(A,B,C.D)

In India Biotyping by this technique was done for the first time in 1993 by Krishna Prakash S and showed that majority belongs to group B. He reported the same finding a decade later also. Similar finding were found by other author's also [102,103].Since this technique is easy to perform, inexpensive and reproducible, in can be incorporated as a daily bench top procedure.

Antibiogram: MRSA can also be typed based on the susceptibility to a range of antibiotics. It is easy to perform but has a poor discriminatory ability and lacks reproducibility.

2. **Genotypic methods:[104,105]**

- Plasmid analysis
- Chromosomal DNA
- Restriction enzyme analysis
- Southern hybridization
- Ribotyping
- Coagulase gene typing
- Protein A gene typing

- RAPD
- Rep-PCR
- Mec-A:Tn 554 probe typing
- Pulse- field gel electrophoresis

RESISTANCE TO OTHER ANTIBIOTICS:

Erythromycin and Clindamycin:

These two are two different classes of antimicrobial agents the inhibit protein synthesis by binding to 50S ribosomal unit of bacterial cell. In *Staphylococci* resistant to both these drugs occur through methylation of their ribosomal target site. Such resistance is mediated by the msrA. Another mechanism of resistance is by inactivation of lincosamides by chemical modification, which is mediated by inuA gene.

The target site modification mechanism also called macrolide lincosamide-streptograminB (MLSB) resistance results in resistance to erythromycin, clindamycin and streptograminB.This may be constitutive or inducible. In constitutive rRNA methylase is always produced, whereas in inducible methylase is produced only in the presence of an inducer.

Invitro, *Staphylococcus aureus* isolates with constitutive resistance are resistant to Erythromycin and clindamycin and isolates with inducible resistance are resistant to erythromycin but appear susceptible to clindamycin

and in vivo therapy with clindamycin may select for erm mutants , and leads to clinical failure.

Invitro induction test can distinguish inducible erm –mediated resistance from those with msr-A mediated resistance. This is known as D-test.[106]

Fluoroquinolones:

Pefloxacin, ciprofloxacin and ofloxacin have activity against Staphylococcus and can be considered for treatment. The target of Fluoroquinolones in *Staphylococci* is topoisomerase IV DNA gyrase.A point mutation in the grl A gene ,that encodes the A subunit of topoisomerase IV leads to resistance. Thus the major limitation of Fluoroquinolones is that resistance develops easily and hence have a limited role as monotherapy in serious infections.[107].

Amino glycosides: Gentamicin, netilmycin and tobramycin are the most effective amino glycosides against Staphylococci. But not effective as a monotherapy due to emergence of resistance. Plasmid mediated resistance develops against Gentamicin.[107,108]]

Vancomycin and Teicoplanin: These are glycopeptides active against MSSA and MRSA. Mi – Na Kim et al 2000 reported a case of Vancomycin intermediate resistance in *Staphylococcus aureus* in Korea

Mupirocin: It is a pseudomonic acid, a natural product of *Pseudomonas fluorescens*. It acts by inhibiting isoleucyl-tRNA synthetase in *Staphylococc*i. It

is used topically to eradicate nasal carriage .Resistance develops due to the presence of an isoleucyl-tRNA synthetase gene located on a conjugative plasmid encoding Gentamicin resistance.[108]

ANTIBIOTIC TREATMENT FOR BACTERIAL INFECTIONS IN DCLD[109]

1. ESBL-producing Enterobacteriaceae- Carbapenems
2. MRSA and VSE – Glycopeptides or Linezolid
3. Uncomplicated urinary tract infections- Nitrofurantoin
4. *Pseudomonas aeruginosa* - Meropenam or Ceftazidime and Ciprofloxacin

Prevention of Infection in Cirrhosis: Antibiotic prophylaxis must be restricted to selected patients at a very high risk for the development of bacterial infections. This restriction of antibiotic usage to prevent the development of antibiotic resistance in DCLD patients and to make these prophylactic strategies cost-effective. Current indications of antibiotic prophylaxis in DCLD are gastrointestinal bleeding, low protein ascites, and previous episode of SBP [110-1

MATERIALS AND METHODS

Place of study:

The study was conducted in the Institute of Microbiology, Madras Medical College in association with various other Departments (Internal Medicine, Gastroenterology, and Hepatology etc) Rajiv Gandhi Government General Hospital (RGGGH), Chennai. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were included in this study.

STUDY DESIGN AND STUDY PERIOD:

The study design was cross sectional study.The study period was from September 2013 to August 2014 (one year).

Study group:

A total of 150 patients of Decompensated Liver disease (DCLD) admitted in various wards of RGGGH with complaints suggestive of bacterial infections like high grade fever, cough with sputum production, altered sensorium, dyspnoea, burning micturition were taken for the study.

ETHICS CONSIDERATION:

This study was conducted after the required approval from the Institutional Ethics Committee. Informed consent was obtained from the study

population. All patients satisfying inclusion criteria were included in this study. Patients were interviewed by a structured questionnaire.

Inclusion criteria:

- Patients of more than 18 years age.
- Patients with Decompensated liver diseases admitted in various wards with signs and symptoms suggestive of bacterial infections.

Exclusion criteria:

- Patients of less than 18 years age.
- Patients with other causes of peritonitis (Tuberculous peritonitis, Malignant ascites)

Collection of data:

Data were collected from patients who satisfied the inclusion criteria, using preformed structured questionnaire. Demographic details like name, age, address, date of admission, diagnosis at admission, habitual history [smoking, alcoholism], past and present Medical history, physical examination findings, nutritional status, underlying illness (Diabetes mellitus, Tuberculosis, malignancy, immunosuppressive drugs Uraemia,) were also included.

Sample collection and Transport:

Under strict aseptic precautions, samples were collected in sterile containers, properly labeled and were transported to the laboratory in appropriate conditions and processed as early as possible.

Types of Samples collected:

1. Ascitic fluid.
2. Blood sample.
3. Urine.
4. Sputum
5. Wound swab

PROCEDURE OF SAMPLE COLLECTION, TRANSPORTATION AND PROCESSING:

COLLECTION OF ASCITIC FLUID / PERITONEAL FLUID:

Under strict aseptic precautions, about 15-20 ml of free fluid in the abdomen (Peritoneal fluid, Ascitic fluid) was aspirated by paracentesis [abdominal tapping] under ultrasound guidance from the patients. Of which 10 ml of ascitic fluid was inoculated in to 50ml of Brain heart infusion broth at the bed side [103,104] and 5 ml of aspirated fluid sent to the pathology department for the estimation of leukocyte count [114].

COLLECTION OF BLOOD:

Two sets of blood samples (20 ml) were drawn over a 24hr period[113].Using a pressure cuff, suitable vein was located in the arm. Deflate the cuff while disinfecting the venepuncture site. Venepuncture site disinfected with 70% alcohol and then with 2% povidone iodine. The disinfecting agent was allowed to act for 1 minute and then 20 ml of blood was drawn through a sterile syringe, 10 ml was added aseptically into 50 ml of Brain heart infusion broth [113,115-116].

Collection of sputum sample [116]:

Just before the collection of sputum sample, the patients were advised to rinse their mouth with water. The patients were requested to take deep breath in and exhale several times and then collected deeply coughed sputum in a clean, dry, wide-necked, leak proof, screw-capped container.

Collection of urine:

Patients were instructed to collect clean catch midstream urine sample in a screw capped wide mouthed sterile container. In case of patients with indwelling catheter, under aseptic precaution sample was collected from sampling port .The sample was immediately transported to the laboratory.

Collection of wound swab:

The wound with surface exudates was washed with sterile saline or 70% alcohol. Tissue or aspirates were preferred over wound swab specimen. The swabs were passed deep into the base of the lesion to firmly sample the fresh border. Two swabs were collected, one for Gram staining and the other for culture.

Specimen processing:

Ascitic fluid

5 – 10 ml of aspirated ascitic and peritoneal fluid observed for macroscopic characteristic features of fluid (color, turbidity, purulent, blood stained).Then, the peritoneal/ascitic fluid was centrifuged at 1500rpm for 10 minutes. The sediment was used for further processing.

Sputum [117-118]:

Sputum sample was homogenized by vortexing and processed further. All the sputum samples were prescreened with Gram's stain, using Bartlett scoring system [119].

Urine:

A loopful of well mixed uncentrifuged urine was placed on a clean glass slide and allowed to air dry, then heat fixed, Gram staining was carried out and examined under oil immersion (X100) field of light microscope and the

number of pus cells, epithelial cells and bacteria was recorded (1 pus cell per low power field corresponds to 3 cell per µl).

A loopful of uncentrifuged urine samples was plated onto blood agar and MacConkey agar. The plates were incubated at 37°C for 24 hours. The number of colonies grown counted and interpretated as colony forming unit per ml (CFU/ml) of urine by multiplying the number of colonies grown by 100. Colony counts exceeding , 10^5 CFU/ml is suggestive of significant bacteriuria

DIRECT MICROSCOPY

All the samples like ascitic fluid, blood, sputum, urine and wound swab were subjected for Gram's staining.

Gram's stain – to detect the presence of bacteria, pus cells, their Gram reaction, morphology and their arrangement.

CULTURE

All the samples were inoculated onto the following culture media by using calibrated loop of 0.01ml and incubated under specified condition

- Nutrient Agar at 37°C incubated for 24 hours.
- 5 % sheep Blood agar in 5-10% CO2 at 37°C incubated for 24 hours
- Chocolate agar in 5-10% CO2 at 37°C incubated for 24 hours.
- MacConkey agar at 37° incubated for 24 hours.

INTERPRETATION:

Interpretation of bacterial cultures: [120]

Bacterial isolates were identified by means of colony morphology, Gram staining, motility, Catalase, oxidase, Coagulase and other biochemical reactions as per standard recommended Microbiological techniques.

ANTIMICROBIAL SENSITIVITY TESTING:

Antibiotic susceptibility testing was performed to know the sensitivity and resistant pattern of all the isolates by the Kirby Bauer method on Cation Adjusted Mueller Hinton agar (Himedia) according to CLSI guidelines [121]. The diameter of zones of inhibition was interpreted according to CLSI standards for each organism. Media and discs were tested for quality control using ATCC strains.

The following standard ATCC strains were used

- *Escherichia coli*-ATCC 25922
- *Staphylococcus aureus*- ATCC 25923
- *Pseudomonas aeruginosa* –ATCC 27853
- *Klebsiella pneumonia*(ESBL) –ATCC 700603

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram negative bacilli were (Himedia).

Antibiotics	Disc content	Inhibition zone in mm		
		Resistance	Intermediate	Sensitive
Amikacin	30µg	14	15-16	17
Ceftazidime	30µg	14	15-17	18
Cefotaxime	30µg	14	15-17	18
Ciprofloxacin	5µg	15	16-20	21
Ofloxacin	5µg	12	13-15	16
Gentamicin	10µg	12	13-14	15
Imipenem	10 µg	13	14-15	16
Piperacillin/ Tazobactum	100/10µg	17	18-20	21

The panel of antibiotics included in the antimicrobial sensitivity testing for Gram positive cocci were (Himedia),

Antibiotics	Disc content	Inhibition zone in mm		
		Resistance	Intermediate	Sensitive
Amikacin	30µg	14	15-16	17
Ciprofloxacin	5µg	15	16-20	21
Cotrimoxazole	1.25/23.75µg	10	11-15	16
Chloramphenicol	30µg	12	13-17	18
Clindamycin	2µg	14	15-20	21
Penicillin	10units	28	-	29
Rifampicin	5µg	16	17-19	20
Erythromycin	15µg	13	14-22	23
Cefoxitin	30µg	21	-	22

Antimicrobial susceptibility testing by Kirby – Bauer Disc Diffusion method:[119]

- Taken 3 to 5 identical colonies from agar plate culture by using sterile bacteriological loop, and transferred into normal saline.
- The colony suspension was matched with 0.5McFarland standard turbidity.
- A sterile non – toxic, non-absorbable cotton swab was dipped into the inoculum.
- Streaking the swab 3 times over the Cation Adjusted Mueller Hinton agar (CAMHA) plate surface, rotating the plate approximately 60 degrees to confirm an equal distribution. Replaced the lid of the dish and allowed 3 to 5 minutes.
- Approximately five antimicrobial discs were placed on the surface of 90mm diameter plate with the help of forceps and incubated at 37^0C overnight.
- After incubation, zone of inhibition was measured in mm from the edge of the disc to the zone edge.

DETECTION OF ß LACTAMASE ENZYME PRODUCTION IN GRAM NEGATIVE BACILLI:

EXTENDED SPECTRUM ß- LACTAMASES (ESBL) DETECTION METHODS:

ESBL's are classified under Bush class A ß- lactamase which are capable of hydrolyzing penicillin – oxyiminocephalosporins and monobactams (Aztreonam) and inhibited by ß-lactamase inhibitors (clavulanic acid, sulbactum and tazobactum) but have no detectable activity against cephamycins or carbapenems (Imipenem, Meropenem).

ESBL Screening method: [122, 123]

Isolates of gram negative bacilli showing the following resistance pattern were considered to be possible ESBL producing strains.

Antibiotic	Zone diameter for possible ESBL producing strain
Ceftazidime(30µg)	≤ 22mm
Cefotaxime(30µg)	≤ 27mm
Ceftriaxone(30µg)	≤ 25mm
Aztreonam(30µg)	≤ 27mm
Cefpodoxime(10 µg)	≤ 17mm

- For *Proteus mirabilis*: Cefpodoxime≤ 22mm

Ceftazidime ≤ 22mm

Cefotaxime ≤ 27mm

Double Disk Diffusion Synergy Test: [124]

The third generation cephalosporin disc and Augmentin disc [Amoxicilin and Clavulanic acid] (20µg/10µg) (Himedia) were kept 30mm apart from centre to centre on Cation Adjusted Mueller Hinton agar (MHA). A clear extension of the edge of the inhibition zone of cephalosporin towards Augmentin disc was interpreted as positive for ESBL production.

Phenotypic Confirmatory Double Disk Test: (PCDDT) [124]

3 – 5 identical colonies were picked from a fresh overnight grown culture with a sterile bacteriological loop and inoculated into 5 ml of normal saline and then turbidity matched with 0.5 McFarland's standard. Lawn culture of the test organism was made on to MHA plate (Himedia, Mumbai), Antibiotic disc Ceftazidime (CAZ 30µg) and Ceftazidime / Clavulanic acid (CAZ/CA 30µg /10µg) (Himedia, Mumbai) were placed 20mm apart onto the plate and incubated at 35^0 C overnight. The difference of ≥ 5mm increase in zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone confirmed an ESBL producing organism.

Detection of carbapenemase production by Modified Hodge test [125]

A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of broth or normal saline was prepared. A 1:10 dilution was streaked as lawn on to a Cation Adjusted Mueller Hinton agar plate. A 10 µg ertapenam susceptibility disk was placed in the center of the test area. Test organism, positive control and negative control were streaked in a straight line from the edge of the disk to the edge of the plate. The plate was incubated overnight at 35±2°C in ambient air for 16–24 hours.

After 24 hrs, MHT Positive test showed a clover leaf-like indentation of the *Escherichia coli* ATCC 25922 growing along the test organism growth streak within the inhibition zone. MHT Negative test showed no growth of the *Escherichia coli* ATCC 25922 along the test organism growth streak within the disc diffusion.

Minimum inhibitory concentration (MIC) for detecting Meropenam

Resistance in Klebsiella oxytoca :

1. Culture media: Cation adjusted Mueller Hinton broth (pH 7.2-7.4).

2. Preparation of antibiotic stock solution:

Antibiotic stock solution can be prepared using the formula,

$$W = \frac{1000}{P} \times V \times C$$

Where P= potency of the antibiotic in relation to the base. (For Meropenem,

P= 675/1000 mg)

V = volume of the stock solution to be prepared (10ml)

C = final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

15.17 mg of drug was mixed with 10ml of distilled water which contains 1024 concentration of drug.

3. Preparation of Antibiotic dilutions:

- Two rows each of 14 sterile test tubes were arranged in the rack (1 row for the test & 2nd row for ATCC control).
- Using sterile pipette, 1ml of MH broth was transferred to all the tubes in the rack.
- From the stock solution 1 ml was transferred to the first tube in each row and mixed well.
- From the first tube 1 ml of the antibiotic solution was transferred to second tube.
- This procedure was repeated till the 14th tube.
- For growth control, 1 ml of inoculum in a test tube without antibiotic should bekept in each row.
- The sterility control for the antibiotic solution was also kept.

4. Inoculum preparation for the test and ATCC control strain :

- 9.9 ml of MH broth was taken in a sterile test tube.
- 0.1ml of 0.5 McFarland turbidity matched test organism was added to broth and mixed well.
- From the above inoculum 1 ml was transferred to each tube containing antibiotic dilutions and also to the control tube using sterile syringe.
- Same procedure was repeated for ATCC control strain.

5. Incubation:

The rack was incubated at 37ºC overnight.

Interpretation:

MIC of ATCC control strain and the test organism were observed.

The lowest concentration of the antibiotic which shows clearing was considered as the MIC for the ATCC strain & for the test isolate.

MIC of meropenam in Enterobacteriaceae:[(126)]

≤ 1µg/ml – Susceptible

2µg/ml – Intermediate

≥4µg/ml - Resistant

DETECTION OF METHICILLIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS*:[127]

- **Cefoxitin** disc diffusion method:
- Media -Muellar Hinton Agar
- Antibiotic disc --Cefoxitin disc 30 µg
- QC Strain ---- *Staphylococcus aureus* ATCC 25923 was used

PROCEDURE:[119,121]

5-6 similar colonies of the organism were picked up from the over night incubated culture plate and inoculated into 5 ml of normal saline and incubated at 37°C for 2 hours. Standard control strain of *Staphylococcus aureus* ATCC 25923 was also inoculated into normal saline and incubated as above. The turbidity of the control and test strains was adjusted to 0.5 McFarland's standard. The test strain was swabbed all over the surface of the Muellar Hinton medium three times, rotating the plate through an angle of 60° after each application and finally, passing the swab round the edge of the agar surface. Similar method was followed for the control strain. A 30µg cefoxitin disc was applied using a sterile forceps at the center of the each plate and pressed gently to ensure even contact with the medium. The plates were incubated at 37°C and readings were taken on the next day.

Interpretation: As per CLSI guidelines 2013

- Zone of inhibition-≥22mm-MSSA
- Zone of inhibition-≤21mm-MRSA

2. Minimum Inhibitory Concentration (MIC) for detecting Vancomycin resistance:

1. Culture media: Cation adjusted Mueller Hinton broth.(pH 7.2-7.4)
2. Preparation of stock antibiotic solution: [131]

Antibiotic stock solution can be prepared using the formula

$$W = \frac{1000}{P} \times V \times C$$

Where P= potency of the antibiotic in relation to the base. (For vancomycin,

P= 950/1000 mg; Himedia)

V = volume of the stock solution to be prepared (10ml)

C =final concentration of the antibiotic solution (1024µg/ml)

W = weight of the antibiotic to be dissolved in the volume V

Scheme of preparing dilution of antibiotics

- Two rows each of 14 sterile test tubes were arranged in the rack (1 row for the test & 2nd row for ATCC control).

- ❖ Using sterile pipette, 1ml of MH broth was transferred to all the tubes in the rack.
- ❖ From the stock solution 1 ml was transferred to the first tube in each row and mixed well.
- ❖ From the first tube 1 ml of the antibiotic solution was transferred to second tube.
- ❖ This procedure was repeated till the 14^{th} tube.
- ❖ For growth control, 1 ml of inoculum in a test tube without antibiotic should be kept in each row.
- ❖ The sterility control for the antibiotic solution was also kept.

b. Inoculum preparation for the test and ATCC control

- ➢ 9.9 ml of MH broth is taken in a uricol container
- ➢ 0.1ml of 0.5 McFarland turbidity matched test organism is added to broth
- ➢ Mixed well and transferred 1 ml of inoculum using 2 ml syringe to each tube containing antibiotic dilutions and also to the control tube.
- ➢ Same procedure repeated for ATCC control strain
- ➢ The rack was incubated at 37ºC for 24 hrs.
- ➢ MIC of ATCC control strain and the test organism was observed.

- The lowest concentration of the antibiotic in which there is no visible growth is considered as the MIC for the ATCC strain & for the test organisms.

Interpretation:

MIC of vancomycin for staphylococcus aureus :[126]

≤ 2µg/ml – Susceptible

4-8µg/ml – Intermediate

≥16µg/ml - Resistant

MIC of vancomycin for Enterococci : [127]

≤ 4µg/ml – Susceptible

8-16µg/ml – Intermediate

≥32µg/ml - Resistant

SEROLOGICAL INVESTIGATIONS:

1. Detection of Complement component C3 level by Enzyme linked immunosorbent assay method
2. Detection of Anti HCV by Enzyme linked immunosorbent assay method.
3. Detection of HBsAg by Enzyme linked immunosorbent assay method.

DETECTION OF COMPLEMENT COMPONENT C3 LEVEL BY ENZYME LINKED IMMUNOSORBENT ASSAY METHOD:

Human Complement component C3 level was detected by Enzyme linked immunosorbent assay in serum by using AssayMax Human Complement C3 ELISA Kit manufactured by ASSAYPRO agencies. The technique of ELISA was performed as per the manufacturer guidelines.

Principle of the Assay

The AssayMax Human Complement C3 ELISA Kit was designed for detection of Human Complement component 3 level in serum and plasma samples. This assay employs a quantitative competitive enzyme immunoassay technique that measures Human Complement component 3 in less than 3 hours. A polyclonal antibody specific for Human Complement component 3 has been pre coated onto micro titre plate. Complement C3 in standards and samples is competed with a biotinylated Complement C3 sandwiched by the immobilized antibody and streptovividin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The colour development is stopped and the intensity of the colour is measured.

Sample collection, preparation and storage:

Sample was collected into a serum separator tube. After clot formation, samples were centrifuged at 3000x g for 10 minutes and removed serum from the supernatant fluid. Then, the sample was diluted 1:800 into EIA

Diluent. The undiluted samples were stored at -20°C or below for up to 3 months.

Reagent preparation:

All reagents are to be brought to room temperature before use and freshly dilute all reagents.

EIA Diluent Concentrate: EIA Diluent Concentrate was diluted with reagent grade water in 1:10 concentration.

Reconstituted the 120µg of Human Complement C3standard with 4ml of EIA Diluent to generate solution of 30 µg / ml and allowed the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepared duplicate or triplicate standard point by serially diluting the standard solution (30 µg / ml). 1:2 with EIA diluent to produce 15, 7.5, 3.75, 1.875, 0.938 and 0.469 µg / ml solutions. EIA diluent serves as the zero standard (0 µg / ml).Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	Complement C3level (µg / ml)
P1	Standard (30 µg / ml)	30.00
P2	1 part of P1 + 1 part of EIA diluents	15.00
P3	1 part of P2 + 1 part of EIA diluents	7.500
P4	1 part of P3 + 1 part of EIA diluents	3.750
P5	1 part of P4 + 1 part of EIA diluents	1.875
P6	1 part of P5 + 1 part of EIA diluents	0.938
P7	1 part of P6 + 1 part of EIA diluents	0.469
P8	EIA diluents	0.000

Biotinylated Human Complement C3 (1x). Reconstituted Biotinylated humaComplement C3 with 4 ml EIA Diluent to produce a working solution.

Assay Procedure

1. Added 25 µl of human complement C3 standard or sample per well and immediately added 25 µl of biotinylated human complement C3 to each well and mixed gently. Covered wells with a sealing tape and incubated for 2 hours.

2. Washed 5 times with 200 µl of wash buffer manually and hit 4 to 5 times on absorbent material to completely remove the liquid.

3. Added 50 µl of Streptavidin Peroxidase conjugate to each well and incubated for 30 minutes and wash 5 times with 200 µl of wash buffer manually.

4. Added 50 µl of Chromogen Substrate per well and incubated for about 20 minutes or until the optimal blue colour density develops.

5. Added 50 µl of stop solution to each well. The Colour changed from blue to yellow.

6. Immediately reading was taken at a wave length of 450 nm on a micro plate reader.

Standard Curve

For generation of a standard curve, plot the graph using the standard concentrations on the X axis and the corresponding mean 450 nm absorbance on the Y axis.

The unknown sample concentration from the standard curve and multiply the value by the dilution factor was determined.

Reference value:

Normal human complement C3 levels range from 0.4 to 2 mg/ml.

DETECTION OF ANTI HCV BY ENZYME LINKED IMMUNOSORBENT ASSAY:

The third generation HCV Microlisa was an in vitro qualitative enzyme linked immunosorbent assay for the detection of antibodies against HCV (Anti-HCVs) in human serum or plasma. This HCV Microlisa Kit was supplied by J. Mira & Co. Pvt .Limited. The technique of ELISA was performed as per the manufacturer guidelines.

PRINCIPLE OF THE ASSAY:

The third generation HCV Microlisa was based on a highly sensitive technique, enzyme linked immunosorbent assay which detects antibodies against HCV in human serum and plasma. The HCV proteins are in serum at levels well below the limits of detection. Thus immune diagnosis of HCV

infection was based on detection of host generated antibodies (Anti-HCVs) to viral proteins.

SPECIMEN COLLECTION & HANDLING

- Blood samples were collected into a test tube and centrifuged at 3000rpm for 10 minutes and the supernatant serum was removed.
- Serums were stored at -20ºC or lower.

TEST PROCEDURE

1. About 100 µl of sample diluents was added to the first well as blank.
2. About 100 µl Negative Control added to each well B-1.
3. Positive Control 100 µl was added to wells C-1, D-1 & E-1
4. In the next step100 µl of sample diluents was added to each well, from F1followed by 10µl of sample was added.
5. The plate was covered with seal and incubated at 37ºC + 2ºC for 30minutes
6. Working wash solution and working conjugate was prepared during incubation of samples.
7. After incubation, the plate was taken from incubator and washed with wash buffer.

8. 100µl of Working Enzyme conjugate was added to each well including A1.

9. The plate was covered and incubated at 37ºC + 2ºC for 30minutes.

10. After incubation, the plate was washed six times with wash buffer.

11. 100µl of Working substrate was added to each well including A1.

12. Aluminium foil was used to cover the plates and incubated at 20-25°C for 30 minutes in dark.

13. Stop solution was added (100 µl) to each well.

14. The Absorbance values were read at 450nm in an Elisa Reader.

CALCULATION OF RESULTS

Test Validity:

- Blank must be < 0.100 in case of differential filter being used.
- PC or PCx must be >0.5. If it is not so, the run is invalid and must be repeated.
- Mean absorbance, PCx = 5.610/3 = 1.870
- NC must be < 0.150

Cut-off value can be determined by using the following formula:

- Cut-off Value = PCx X 0.23

INTERPRETATION OF RESULTS

The absorbance of the unknown sample is compared with the calculated cut-off value.

Test specimens with absorbance (O.D.) value less than cut-off value were regarded non-reactive and were considered as negative for Anti HCV.

Test specimens with absorbance (O.D.) value greater than or equal to cut off value were considered reactive for Anti HCV MICROLISA.

DETECTION OF HEPATITIS B SURFACE ANTIGEN (HBSAG) BY ENZYME LINKED IMMUNOSORBENT ASSAY:

Detection of Hepatitis B surface Antigen (HBsAg) in human serum or plasma by enzyme linked immunosorbent assay with Hepalisa kit. This Hepalisa Kit was supplied by J. Mitra & Co. Pvt .Limited. The technique of Elisa was performed as per the manufacturer guidelines.

PRINCIPLE OF THE ASSAY:

HEPALISA is a solid phase enzyme linked immunosorbent assay (ELISA) based on the "Direct Sandwich" principle. The microwells were coated with Monoclonal antibodies with high reactivity for HBsAg. The samples were added in the wells followed by addition of enzyme conjugate (polyclonal antibodies linked to Horseradish Peroxidase (HRPO). A sandwich complex was formed in the well wherein HBsAg (from serum sample) was "trapped" or "sandwiched" between the antibody and antibody HRPO

conjugate. Unbound conjugate was washed off with wash buffer. The amount of bound peroxidase was proportional to the concentration of HBsAg present in the sample. Upon addition of the substrate buffer and chromogen, a blue colour develops. The intensity of developed blue colour was proportional to the concentration of HBsAg present in the sample.

SPECIMEN COLLECTION & HANDLING

- Blood samples were collected into a test tube and centrifuged at 3000rpm for 10 minutes and the supernatant serum was removed.
- Serums were stored at -20ºC or lower.

TEST PROCEDURE

1. The first well A-1 as blank.
2. About 100 µl Negative Control added to each well B-1and C-1respectively.
3. Positive Control 100 µl was added to wells, D-1 & E-1
4. In the next step100 µl of sample was added to each well.
5. Added 50µl of Enzyme conjugate to each well except A1
6. The plate was covered with seal and incubated at 37ºC + 1ºC for 60minutes

7. After incubation, the plate was taken from incubator and washed with wash buffer.

8. 100µl of Working substrate was added to each well includingA1.

9. Aluminium foil was used to cover the plates and incubated at 20-25°C for 30 minutes in dark.

10. Stop solution was added (100µl) to each well.

11. The Absorbance values were read at 450nm in an Elisa Reader.

CALCULATION OF RESULTS

Test Validity:

- Blank must be < 0.100 in case of differential filter being used.
- PC or PCx must be >0.5. If it is not so, the run is invalid and must be repeated.
- Mean absorbance, PCx = 1.430+1.500=2.930÷2= 1.465
- Mean absorbance, PCx= 1.465.
- NC must be < 0.150
- NC=0.012+0.010=0.022
- Mean absorbance, NCx= 0.022/2= 0.011

Cut-off value can be determined by using the following formula:

- Cut-off Value = NCx + 0.1

INTERPRETATION OF RESULTS

The absorbance of the unknown sample is compared with the calculated cut-off value.

Test specimens with absorbance (O.D.) value less than cut-off value were regarded non-reactive and were considered as negative for HBsAg.

Test specimens with absorbance (O.D.) value greater than or equal to cut off value were considered reactive for HBsAg.

RESULTS

Total number of 150 patients with DCLD with signs and symptoms of bacterial infections (who satisfied the inclusion criteria) were included in this study from September 2013 to August 2014 (one year)

TABLE 1: ANALYSIS OF SEX DISTRIBUTION IN DCLD PATIENTS (n=150)

SEX	No. of patients
Male	145 (97%)
Female	5(3%)
Total	150(100%)

Among 150 patients (n = 150), 97% were males and 3% were females. Males were predominantly affected groups than female in DCLD. It might be due to more distribution of male patients with DCLD than female.

CHART1: ANALYSIS OF SEX DISTRIBUTION IN DCLD PATIENTS

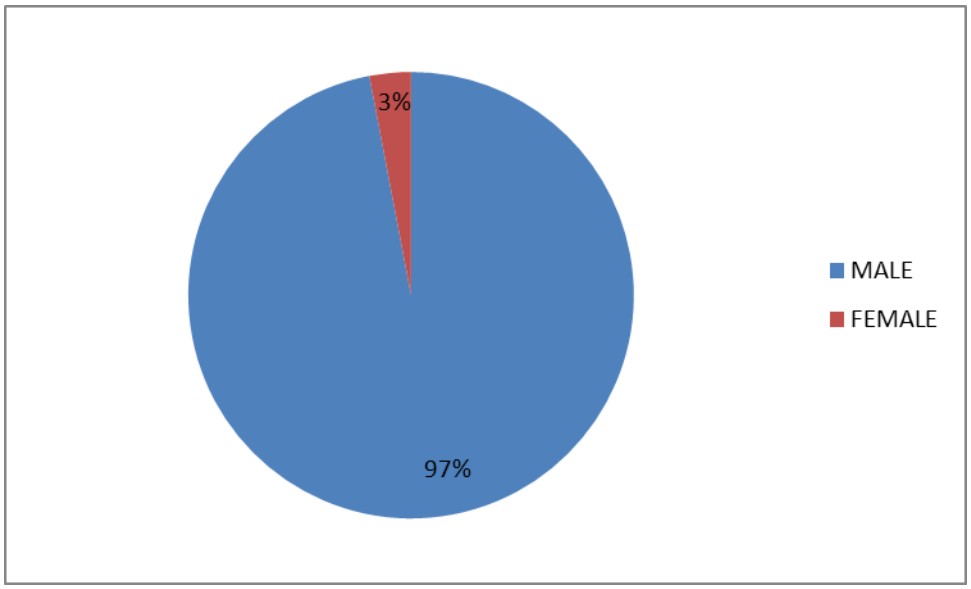


TABLE 2: ANALYSIS OF AGE DISTRIBUTION OF ALL PATIENTS WITH DCLD(n=150)

S.no.	Age	Male	Female	Total
1	≤ 30years	8(5.33%)	1(0.67%)	9 (6%)
2	31-40 years	44(29.33%)	-	44(29.33%)
3	41-50 years	49(32.67%)	1(0.66%)	50(33.33%)
4	51-60 years	37(24.67%)	2(1.33%)	39(26%)
5	≥60years	7(4.66%)	1(0.67%)	8(5.33%)
	Total	145(97%)	5(3%)	150(100%)

Majority of patients belonged to 41-50years (33.33%) of age group followed by 31-40 years (29.33%), 51-60 years (26%) of age. Age groups of less than 30years and more than 60 years were less commonly involved in this study.

CHART 2: ANALYSIS OF AGE DISTRIBUTION IN PATIENTS WITH DCLD

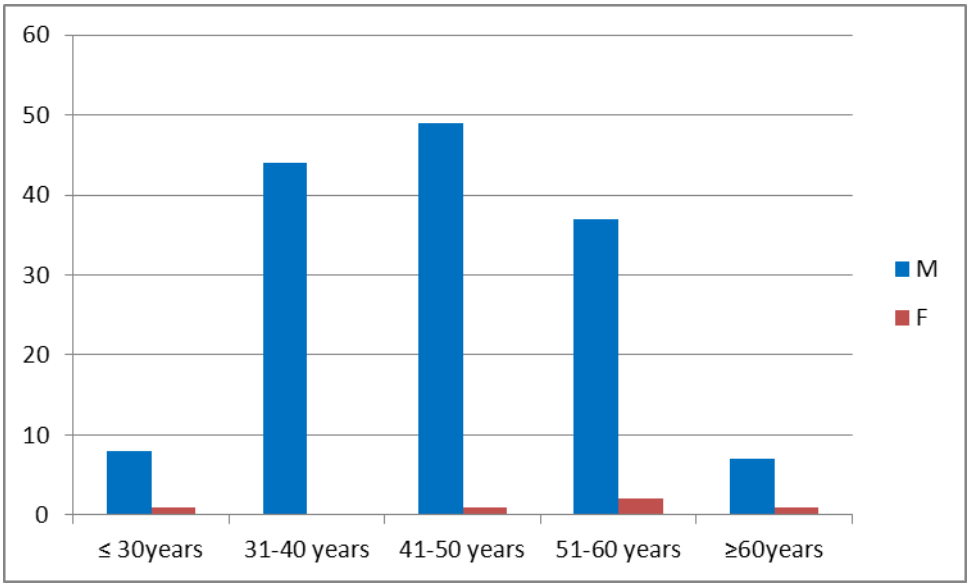


TABLE 3:SAMPLES DISTRIBUTION AND BACTERIAL INFECTIONS IN DCLD PATIENTS (n = 150)

S. no	Type of sample	Number of sample (%) (n=150)	Culture positive (%) (n=81)
1	Ascitic fluid –Spontaneous bacterial peritonitis	59 (39.33%)	22 (27%)
2	Urine- UTI	44 (29.33%)	21 (26%)
3	Sputum- Pneumonia	27 (18.00%)	13 (16%)
4	Wound swab- Skin infections	20 (13.33%)	10 (12%)
5	Blood- Spontaneous bacteraemia	150 (100%)	15 (19%)

Out of 150 patients, the common samples received and processed were ascitic fluid (39.33%), followed by urine (29.33%), sputum (18%) and wound swab (13.33%). Blood sample was collected from all the 150 (100%) patients.

Among 150 patients of DCLD, 81(54%) patients were culture positive. Of which, spontaneous bacterial peritonitis 22(27%) were common, followed by urinary tract infection 21(26%), spontaneous bacteraemia 15(19%), pneumonia 13(16%) and skin and soft tissues infection 10(12%).

CHART 3: DISTRIBUTION OF CULTURE POSITIVITY IN VARIOUS TYPES OF SAMPLES IN DCLD PATIENTS

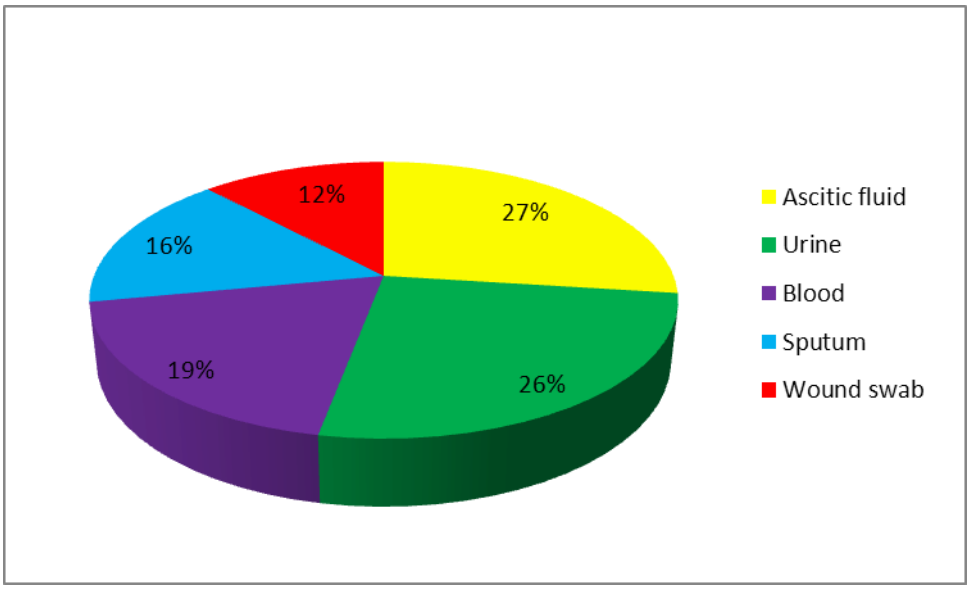


TABLE- 4: CAUSES OF DCLD

S. no.	Cause	Percentage
1	Alcoholic liver disease	126(84%)
2	Viral hepatitis	15(10%)
3	Both Alcoholic liver diseases and Viral hepatitis	8(5.34%)
4	Cryptogenic	1(0.66%)
	Total	**150 (100%)**

The main causes of decompensated liver disease was Alcoholic liver disease(84%), followed by viral hepatitis (10%), both alcoholic liver diseases and viral hepatitis together (5.34%) and Cryptogenic causes(0.66%).

CHART-4: PERCENTAGE OF CAUSES OF DCLD

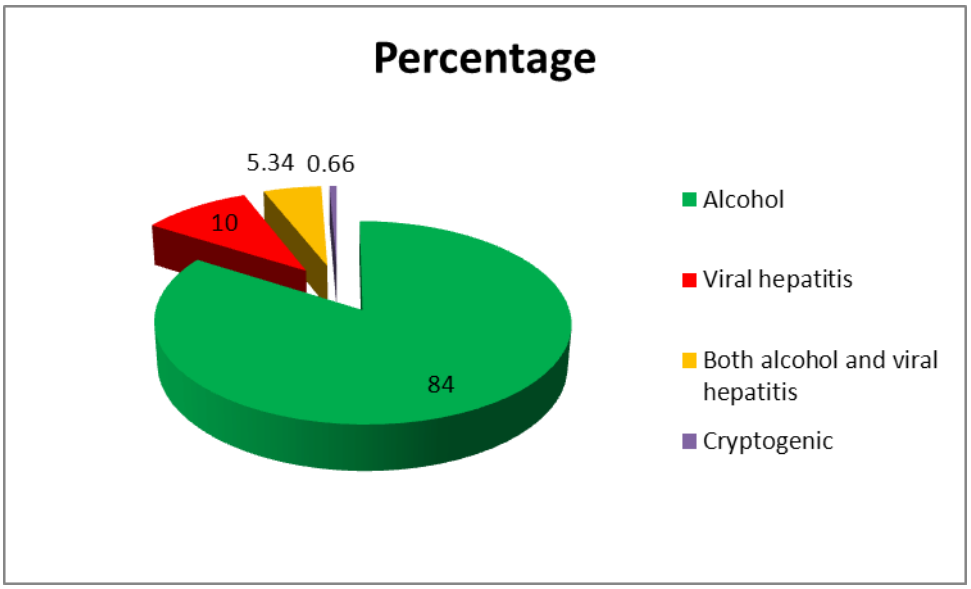


TABLE 5: ANALYSIS OF CULTURE POSITIVITY IN VARIOUS AGE GROUP (n = 150)

S.No.	Age	CULTURE POSITIVE	CULTURE NEGATIVE	TOTAL
1	≤ 30years	4[2.67%]	5 [3.33%]	9 [6%]
2	31-40 years	20 [13.33%]	24 [16%]	44 [29.33%]
3	41-50 years	32 [21.33%]	18 [12%]	50 [33.33%]
4	51-60 years	20 [13.33%]	19 [12.66%]	39 [26%]
5	≥60years	5 [3.34%]	3 [2%]	8 [5.34%]
	Total	**81[54%]**	**69 [46%]**	**150 [100%]**

The age groups 41 to 50 years were most commonly affected with bacterial infections, followed by 51- 60 years and 31- 40 years.

CHART-5: ANALYSIS OF CULTURE POSITIVITY IN VARIOUS AGE GROUP (n = 15O)

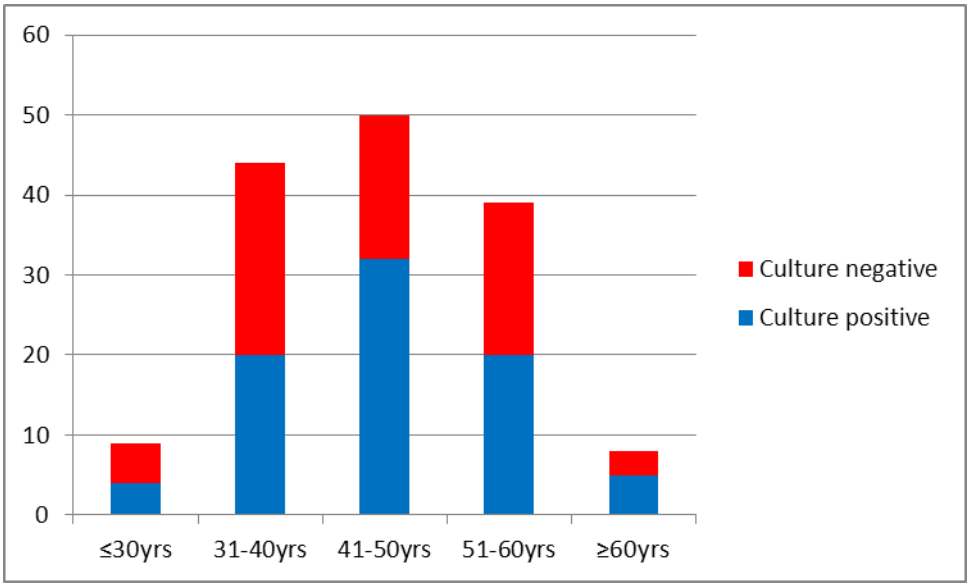


TABLE 6 : DETECTION OF GNB AND GPC IN VARIOUS TYPES OF BACTERIAL INFECTIONS IN DCLD

Type of infections in DCLD (n=11)	GRAM POSITIVE COCCI	GRAM NEGATIVE BACILLI
Spontaneous bacterial peritonitis (n=22)	6 (27%)	16(73%)
Urinary tract infections (n=21)	1(5%)	20(95%)
Spontaneous bacteraemia (n=15)	7(47%)	8(53%)
Pneumonia(n=13)	0	13(100%)
Skin & soft tissues infections (n=10)	4(40%)	6(60%)
Total 81	**18 (22%)**	**63(78%)**

In this study, out of 81 culture positive isolates, 63(78%) were Gram Negative bacilli and 18 (22%) were Gram Positive cocci. (P value = 0.005 – Highly significant).

CHART-6 : DETECTION OF GNB AND GPC IN VARIOUS TYPES OF BACTERIAL INFECTIONS IN DCLD

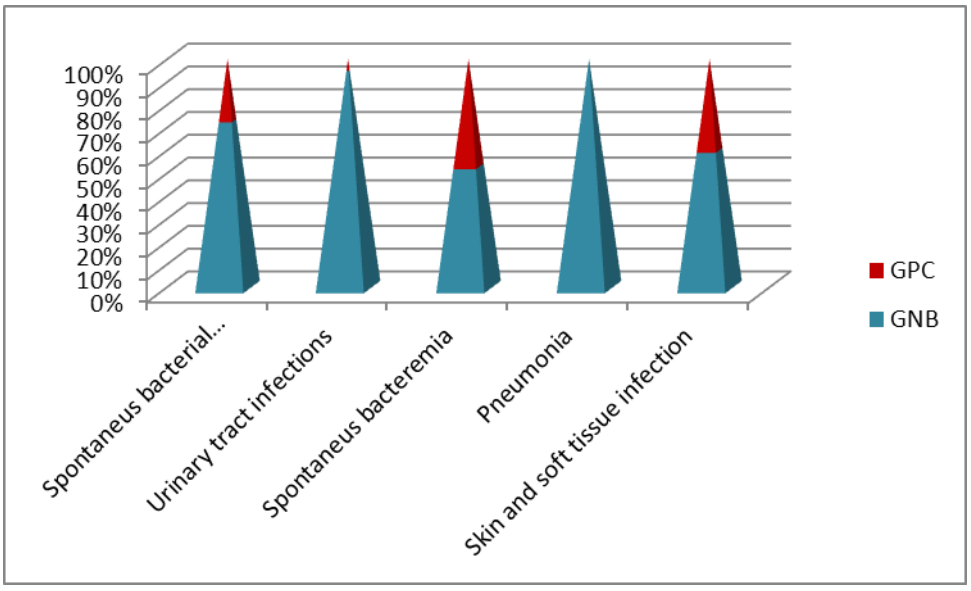


TABLE 7:ANALYSIS OF BACTERIAL ISOLATES FROM ASCITIC FLUID IN PATIENTS OF SBP WITH DCLD(n =22)

S.No.	Organisms	Number [n = 22]	Percentage
1	*Escherichia coli*	7	31.82%
2	*Klebsiella pneumoniae*	3	13.63%
3	*Klebsiella oxytoca*	1	4.54%
4	*Proteus mirabilis*	1	4.54%
5	*Enterobacter cloacae*	1	4.54%
6	*Citrobacter koseri*	1	4.54%
7	*Pseudomonas aeruginosa*	1	4.54%
8	*Acinetobacter baumanii*	1	4.54%
9	*Staphylococcus aureus*	4	18.18%
10	*Enterococcus faecalis*	2	9.09%
	TOTAL	**22**	**100%**

In patients with spontaneous bacterial peritonitis (SBP) the most frequently isolated organisms were *E. coli* (31.82%),*Staphylococcus aureus(18.18%), Klebsiella pneumoniae(13.63%)* and *Enterococcus faecalis(9.09%).*

CHART-7:ANALYSIS OF BACTERIAL ISOLATES FROM ASCITIC FLUID IN PATIENTS OF SBP WITH DCLD

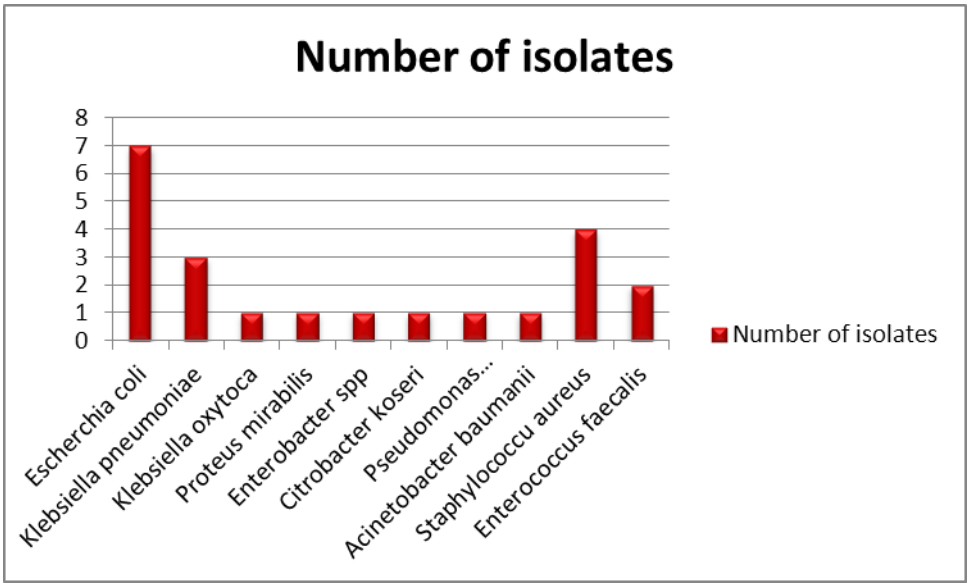


TABLE 8:ANALYSIS OF BACTERIAL ISOLATES FROM URINE IN PATIENTS OF URINARY TRACT INFECTIONS WITH DCLD (n =21)

S.no.	Organisms	Number (n=21)	Percentage
1	*Escherichia coli*	8	38.09%
2	*Klebsiella pneumoniae*	3	14.28%
3	*Klebsiella oxytoca*	6	28.57%
4	*Enterobacter cloacae*	1	4.76%
5	*Acinetobacter baumanii*	2	9.52%
6	*Enterococcus faecalis*	1	4.76%
	Total	**21**	**100%**

In urine samples, 21were culture positive. Of which *Escherichia coli* was the most common isolate (38.09%) followed by *Klebsiella oxytoca* (28.57%), *Klebsiella pneumoniae* (14.28%), *Acinetobacter baumanii* (9.52%) and *Enterococcus faecalis* (4.76%)

CHART-8:ANALYSIS OF BACTERIAL ISOLATES FROM URINE IN PATIENTS OF URINARY TRACT INFECTIONS WITH DCLD

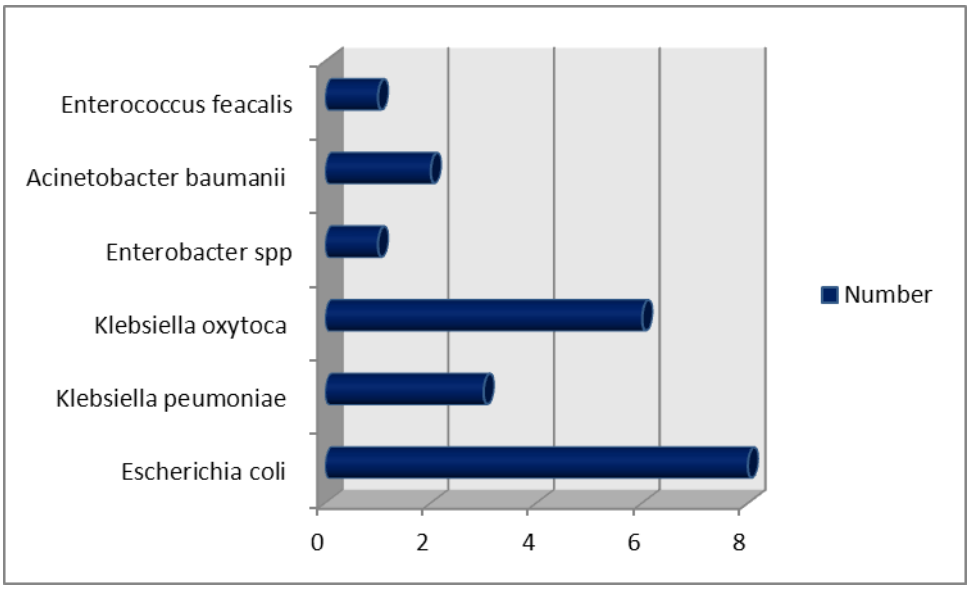


TABLE 9:ANALYSIS OF BACTERIAL ISOLATES FROM BLOOD IN PATIENTS OF BACTERIMIA WITH DCLD(n =15)

S.No.	Organisms	Number [n =15]	Percentage
1	*Escherichia coli*	2	13.33%
2	*Klebsiella pneumoniae*	4	26.33%
3	*Pseudomonas aeruginosa*	2	13.33%
4	*Staphylococcus aureus*	3	20.00%
5	*Streptococcus viridians*	2	13.33%
6	*Staphylococcus epidermidis*	2	13.33%
	Total	**15**	**100%**

Among 150 blood samples, 15 samples were culture positive. Of which *Klebsiella pneumoniae* (26.33%) was the most common isolate followed by *Staphylococcus aureus* (20.00%), *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus viridians*. Two isolates (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were isolated from ascitic fluid.

CHART-9:ANALYSIS OF BACTERIAL ISOLATES FROM BLOOD IN PATIENTS OF BACTERAEMIA WITH DCLD

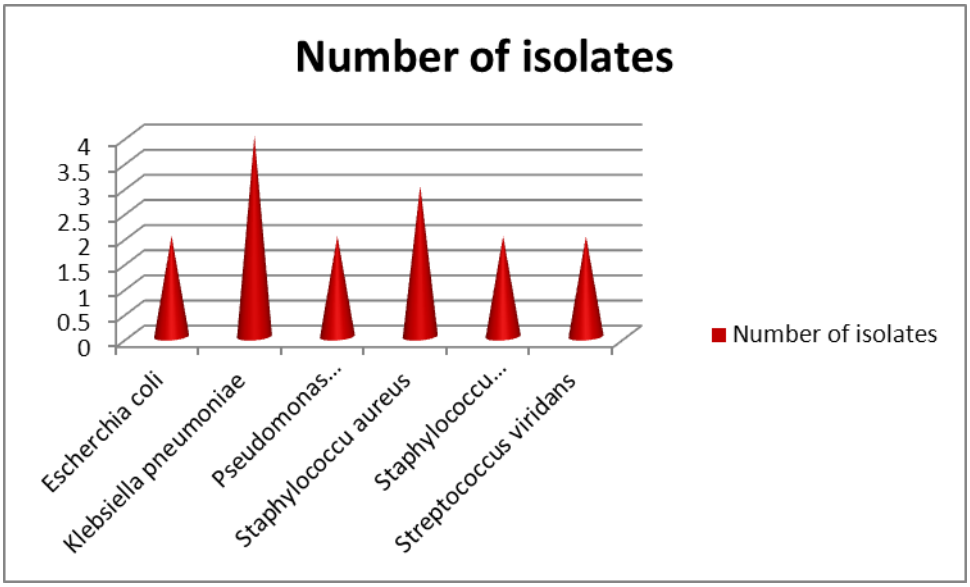


TABLE 10:ANALYSIS OF BACTERIAL ISOLATES FROM SPUTUM IN PATIENTS OF PNEUMONIA WITH DCLD(n =13)

S.No.	Organisms	Number [n =13]	Percentage
1	*Escherichia coli*	1	7.69%
2	*Klebsiella pneumoniae*	3	23.07%
3	*Klebsiella oxytoca*	6	46.14%
4	*Pseudomonas aeruginosa*	3	23.07%
	Total	**13**	**100%**

Out of 27 sputum samples, 13 samples were culture positive. *Klebsiella oxytoca* (46.14%) was the most common isolate followed by *Klebsiella pneumoniae* (23.07%) *Pseudomonas aeruginosa* (23.07%) and *Escherichia coli* (7.69%).

CHART-10:ANALYSIS OF BACTERIAL ISOLATES FROM SPUTUM IN PATIENTS OF PNEUMONIA WITH DCLD

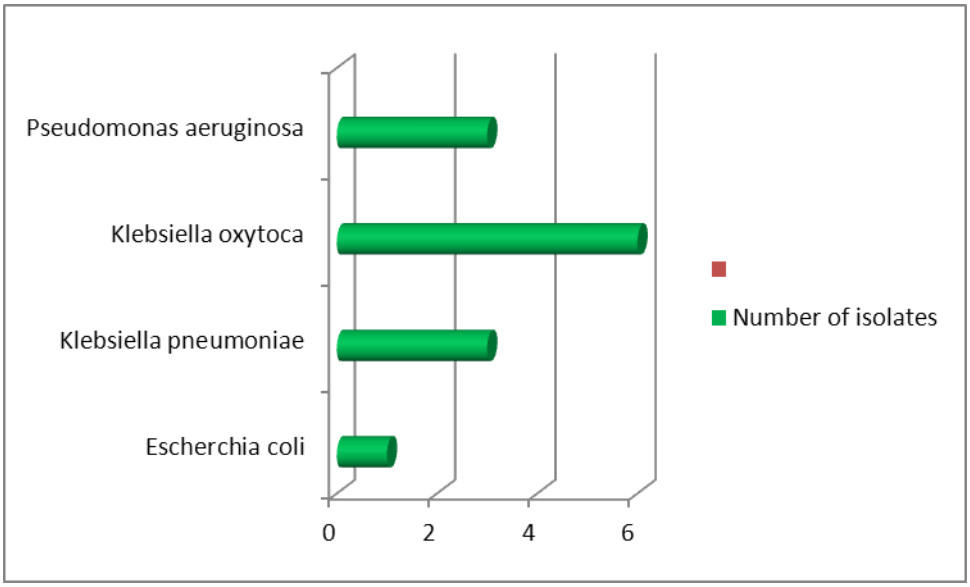


TABLE 11:ANALYSIS OF BACTERIAL ISOLATES FROM WOUND SWABS IN PATIENTS OF SKIN INFECTION WITH DCLD(n =10)

S.No.	Organisms	Number [n =10]	Percentage
1	*Escherichia coli*	1	10%
2	*Klebsiella pneumoniae*	1	10%
3	*Proteus vulgaris*	3	30%
3	*Pseudomonas aeruginosa*	1	10%
4	*Staphylococcus aureus*	2	20%
6	*Staphylococcus epidermidis*	2	20%
	Total	**10**	**100%**

Out of 18 wound swab, 10 were culture positive. *Proteus vulgaris (30%)* was the main isolates followed by *Staphylococcus aureus* (20%), *Staphylococcus epidermidis (20%), Escherichia coli* (10%) *and Klebsiella pneumoniae* (10%).

CHART-11:ANALYSIS OF BACTERIAL ISOLATES FROM WOUND SWAB IN PATIENTS OF SKIN INFECTION WITH DCLD

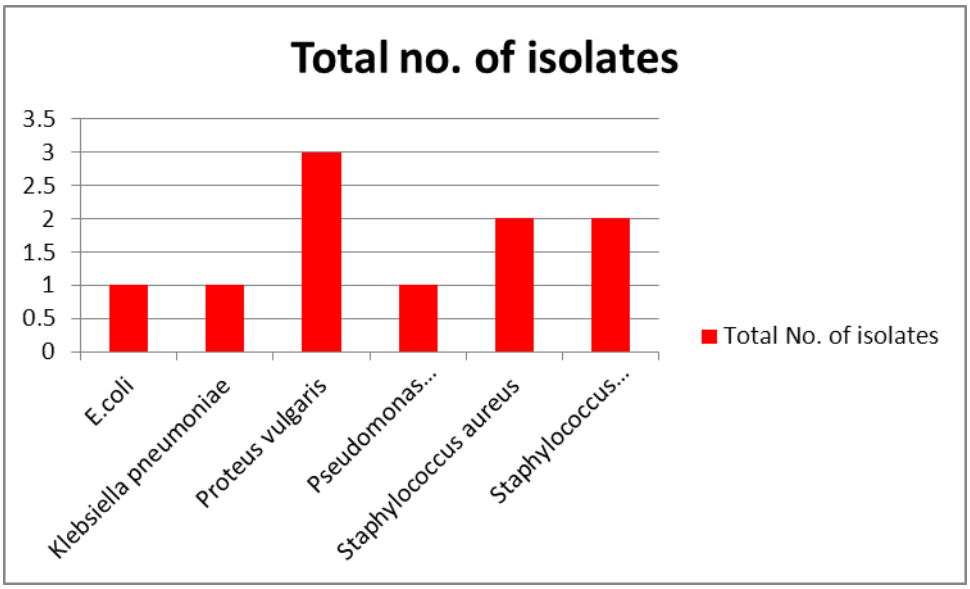


TABLE 12: ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR GRAM NEGATIVE ISOLATES IN SBP FROM PATIENTS WITH DCLD. (n=16)

Name of the Antibiotics	*E.coli* (n=7)		*Klebsiella pneumoniae* (n=3)		*Klebsiella oxytoca* (n=1)		*Enterobacter cloacae* (n=1)		*Citrobacter koseri* (n=1)		*Proteus mirabilis* (n=1)		*P.aeruginosa* (n=1)		*Acinetobacter baumanii* (n=1)	
	no	%	no	%	no	%	no	%	no	%	no	%	no	%	no	%
Amikacin	6	86	2	67	1	100	1	100	1	100	1	100	1	100	1	100
Gentamicin	5	71	1	33	1	100	0	0	0	100	1	100	1	100	1	100
Ceftazidime	3	28	1	33	1	100	0	0	1	100	1	100	1	100	1	100
Cefotaxime	3	28	1	33	1	100	0	0	1	100	1	100	-	-	1	100
Ciprofloxacin	7	100	3	100	1	100	1	100	1	100	1	100	0	0	1	100
Ampicillin	7	100	3	100	1	100	0	0	1	100	1	100	0	0	1	100
Cotrimoxazole	6	86	3	100	1	100	1	100	1	100	1	100	0	0	1	100
Piperacillin / Tazobactum	7	100	3	100	1	100	1	100	1	100	1	100	1	100	1	100
Imipenem	7	100	3	100	1	100	1	100	1	100	1	100	1	100	1	100
Tetracycline	7	100	2	67	1	100	1	100	1	100	1	100	1	100	1	100

All the GNB isolated from spontaneous bacterial peritonitis were100% sensitive to imipenam Mostly sensitive to Amino glycosides and fluoroquinolones.

Out of 16 GNB, 56% only showed sensitivity to third generation cephalosporins. *Pseudomonas aeruginosa* isolate as showed 100% sensitivity to ceftazidime. *Acinetobacter baumanii* showed 100% sensitivity to all other drugs used against for GNB.

TABLE 13: ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR GRAM POSITIVE COCCI IN SBP PATIENTS WITH DCLD.(n=6)

Name of the Antibiotics	***Staphylococcus aureus*** **(n=4)**		***Enterococcus faecalis*** **(n=2)**	
	no	**%**	**no**	**%**
Amikacin	4	100	-	-
Gentamicin	3	75	-	-
Ciprofloxacin	3	75	-	-
Ampicillin	3	75	2	100
Cotrimoxazole	3	75	-	-
Tetracycline	3	75	0	0
Chloramphenical	-	-	2	100
Amoxycillin	-	-	-	-
Erythromycin	2	50	2	100
Cefoxitin	2	50	-	-
HL Gentamicin	-	-	2	100
Penicillin	3	75	2	100
Vancomycin (MIC)	2	50	1	50

- 50% of *Staphylococcus aureus* were Methicillin sensitive.
- 50% of *Enterococcus faecalis* were vancomycin sensitive Enterococci (VSE). *Enterococcus faecalis* were 100% sensitive to all other drugs

TABLE 14: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF URINARY ISOLATES FROM DCLD PATIENTS (n=20)

Name of the Antibiotics	*E.coli* (n=8)		Klebsiella pneumoniae (n=3)		Klebsiella oxytoca (n=6)		Enterobacter cloacae (n=1)		Acinetobacter baumanii (n=2)	
	no	%	no	%	no	%	no	%	no	%
Amikacin	7	88	3	100	4	67	1	100	1	50
Gentamicin	4	50	3	100	3	50	1	100	1	50
Ceftazidime	3	37	1	33	1	17	1	100	0	0
Cefotaxime	3	37	1	33	1	17	1	100	0	0
Ciprofloxacin	5	62	3	100	3	50	1	100	1	50
Norfloxacin	4	50	1	33	2	33	1	100	0	0
Nitrofurantoin	1	13	2	33	2	33	1	100	2	100
Cotrimoxazole	6	75	3	100	4	67	1	100	2	100
Piperacillin / Tazobactum	5	62	3	100	5	83	1	100	2	100
Imipenem	8	100	3	100	6	100	1	100	2	100
Chloramphenical	-	-	3	100	5	83	1	100	-	-
Tetracycline	8	100	3	100	6	100	1	100	2	100

- Among urinary tract infections, all the GNB were 30% sensitive to third generation cephalosporins.

- *Klebsiella pneumoniae* was 100% sensitive to amino glycosides fluoroquinolones, carbapenems and piperacillin / Tazobactum.

- *Enterobacter cloacae* was 100% sensitive to all other drugs.

TABLE 15: ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR ENTEROCOCCUS FAECALIS IN URINARY ISOLATES FROM DCLD PATIENTS (n=1)

Name of the Antibiotics	***Enterococcus faecalis* (n=1)**	
	no	**%**
Tetracycline	1	100
HL Gentamicin	0	0
Penicillin	0	0
Vancomycin	1	100
Ampicillin	1	100
Norfloxacin	1	100
Nitrofurantoin	0	0

- *Enterococcus faecalis* was 100% sensitive to vancomycin.

TABLE 16: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ISOLATES FROM BLOOD OF DCLD PATIENTS (n=8)

Name of the Antibiotics	*E.coli*(n=2)		*Klebsiella pneumoniae*(n=4)		*Pseudomonas aeruginosa* (n=2)	
	no	%	no	%	no	%
Amikacin	2	100	3	75	2	100
Gentamicin	1	50	2	50	2	100
Ceftazidime	1	50	3	75	1	50
Cefotaxime	1	50	3	75	-	-
Ciprofloxacin	2	100	2	50	0	0
Cotrimoxazole	2	100	2	50	-	-
Piperacillin / Tazobactum	2	100	4	100	2	100
Imipenem	2	100	4	100	2	100
Tetracycline	2	100	4	100	2	100

All the GNB isolated from blood showed 100% sensitive to amino glycosides, carbapenems and piperacillin / Tazobactum.

Out of 8 GNB in blood culture, 2 isolates (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were isolated from ascitic fluid with same sensitivity pattern.

TABLE 17: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE ISOLATES FROM BLOOD OF DCLD PATIENTS (n=7)

Name of the Antibiotics	*Strep. viridans* (n=2)		*S. aureus* (n=3)		*S.epidermidis* (n=2)	
	no	%	no	%	no	%
Amikacin	-	-	3	100	2	100
Gentamicin	-	-	2	67	1	50
Ciprofloxacin	2	100	3	100	2	100
Ampicillin	2	100	2	67	1	50
Cotrimoxazole	2	100	2	67	2	100
Chloramphenical	1	50	-	-	-	-
Tetracycline	1	50	2	67	1	50
Amoxycillin	-	-	-	-	-	-
Erythromycin	2	100	1	33	2	100
Cefoxitin			2	67	2	100
Penicillin	2	100	1	33	2	100
Vancomycin (MIC)	1	50	2	100	2	100

- In Blood culture, *Streptococcus viridians* showed 100% sensitive to ciprofloxacin, ampicillin, cotrimoxazole and erythromycin.
- *Staphylococcus aureus* were 67% sensitive to methicillin.
- *Staphylococcus epidermidis* were showed 100% sensitive to methicillin, vancomycin and amino glycosides.
- All the GPC were 100% sensitive to fluoroquinolones.

TABLE 18: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ISOLATES FROM PNEUMONIA OF DCLD PATIENTS (n= 13)

Name of the Antibiotics	***E.coli* (n=1)**		***Klebsiella pneumoniae* (n=3)**		***Klebsiella oxytoca* (n=6)**		***Pseudomonas aeruginosa* (n=3)**	
	no	**%**	**no**	**%**	**no**	**%**	**no**	**%**
Amikacin	1	100	1	33	4	67	3	100
Gentamicin	0	0	1	33	3	50	3	100
Ceftazidime	0	0	1	33	1	16	2	67
Cefotaxime	0	0	1	33	1	16	2	67
Ciprofloxacin	0	0	2	67	3	50	2	67
Ampicillin	1	100	2	67	2	67	-	-
Cotrimoxazole	1	100	2	67	3	50	3	100
Piperacillin / Tazobactum	1	100	3	100	5	83	2	67
Imipenem	1	100	3	100	5	83	3	100
Tetracycline	1	100	3	100	5	83	3	100

- In the sputum samples, among 13 GNB, 31% sensitive to third generation cephalosporins.
- All the GNB were 100% sensitive to carbapenem except one *Klebsiella oxytoca* which was resistant to carbapenem.
- No GPC was isolated from sputum samples.

TABLE 19: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ISOLATES FROM WOUND SWAB OF DCLD PATIENTS (n=6)

Name of the Antibiotics	*E.coli* (n=1)		*Klebsiella pneumoniae* (n=1)		*Proteus vulgaris* (n=3)		*Pseudomonas aeruginosa* (n=1)	
	no	%	no	%	no	%	no	%
Amikacin	1	100	0	0	2	67	1	100
Gentamicin	1	100	1	100	2	67	1	100
Ceftazidime	0	0	0	0	0	0	1	100
Cefotaxime	0	0	0	0	0	0	0	0
Ciprofloxacin	0	0	0	0	2	67	1	100
Ampicillin	1	100	0	0	3	100	1	100
Cotrimoxazole	1	100	1	100	2	67	1	100
Piperacillin / Tazobactum	1	100	1	100	3	100	1	100
Imipenem	1	100	1	100	3	100	1	100
Tetracycline	1	100	1	100	3	100	1	100

- *Pseudomonas aeruginosa* was sensitive to all drugs.
- Among GNB, 17 % sensitive to third generation cephalosporin.
- *E.coli* was 100% sensitive to amino glycosides, piperacillin / tazobactum, Imipenem and gentamicin.
- All the GNB were 100% piperacillin / tazobactum, imipenem and tetracycline

TABLE 20: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE ISOLATES FROM WOUND SWAB OF DCLD PATIENTS (n=4)

Name of the Antibiotics	***Staphylococcus aureus*(n=2)**		***Staphylococcus epidermidis* (n=2)**	
	no	**%**	**no**	**%**
Amikacin	2	100	1	50
Gentamicin	1	50	1	50
Ciprofloxacin	2	100	1	50
Ampicillin	1	50	1	50
Cotrimoxazole	2	100	2	100
Tetracycline	2	100	2	100
Erythromycin	2	100	2	100
Cefoxitin	1	50	2	100
Penicillin	1	50	2	100
Vancomycin (MIC)	2	100	2	100

- In our study, 50% of *Staphylococcus aureus* were Methicillin sensitive.
- All the GPC were 100% sensitive to vancomycin

TABLE 21: DETECTION OF ESBL PRODUCTION FROM GRAM NEGATIVE ISOLATES IN DCLD PATIENTS

S.no.	Type of infections (GNB n=63)	Screening Test		DDST		PCDDT	
		no.	%	no.	%	no.	%
1.	SBP [Total no of GNB=16]	7	43.75	5	31.25	4	25
2.	Urinary tract infections [Total no of GNB = 20]	14	70	12	60	11	55
3.	Spontaneous Bacteraemia [Total no of GNB = 8]	2	25	2	25	2	25
4	Pneumonia [Total no of GNB = 13]	8	61.53	6	46.15	5	38
5	Skin & soft tissues infections [Total no of GNB = 6]	4	66.66	4	66.66	3	50
	Total	**35**	**56%**	**27**	**43%**	**25**	**40%**

DDST – Double disk diffusion synergy test

PCDDT – Phenotypic Confirmatory disk diffusion test

Majority of ESBL isolates 55% (11/20) were from urinary tract infections followed by skin & soft tissues infections 50% (3/6), pneumonia 38% (5/13), spontaneous bacterial peritonitis 25% (4/16) and spontaneous bacteraemia 25% (2/8).

CHART-12: DETECTION OF ESBL PRODUCTION IN DIFFERENT INFECTIONS OF DCLD

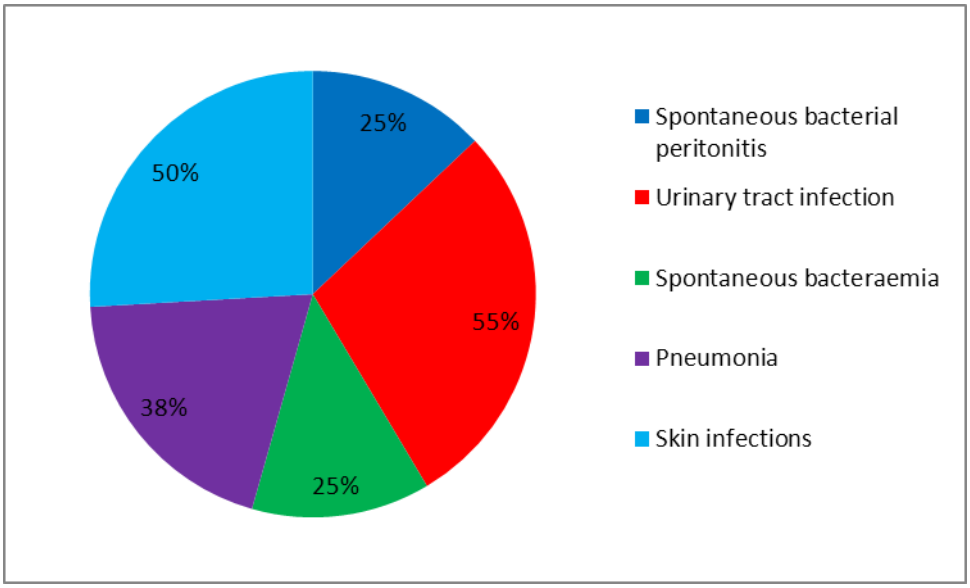


TABLE 22 : INTERPRETATION OF MIC OF MEROPENEM FOR CARBAPENEMASE PRODUCING ENTEROBACTERIACEAE BY MACROBROTH DILUTION METHOD

Name of isolates	MIC value	Interpretation
Klebsiella oxytoca (1)	≥16µg/ml	Resistant

INTERPRETATION:

≤ 1µg/ml – Susceptible

2µg/ml – Intermediate

≥4µg/ml – Resistant.

TABLE 23: DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN VARIOUS TYPES OF BACTERIAL INFECTIONS IN DCLD (n=13)

Type of infections in DCLD (Total no of GPC -13)	MRSA	
	Resistance	%
Spontaneous bacterial peritonitis (n= 4)	2	50
urinary tract infections (n=0)	-	-
Spontaneous bacteraemia (n=5)	1	20
Pneumonia(n=0)	-	-
Skin & soft tissues infections (n=4)	1	25

Total of 13 GPC, 9 (69%) were Methicillin sensitive and 4(31%) were Methicillin resistant, of which 2(50%) of MRSA were isolated from spontaneous bacterial peritonitis, 1(25%) MRSA from spontaneous bacteraemia and 1(25%) MRSA from Skin & soft tissues infections.

CHART-13: DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN VARIOUS TYPES OF BACTERIAL INFECTIONS IN DCLD

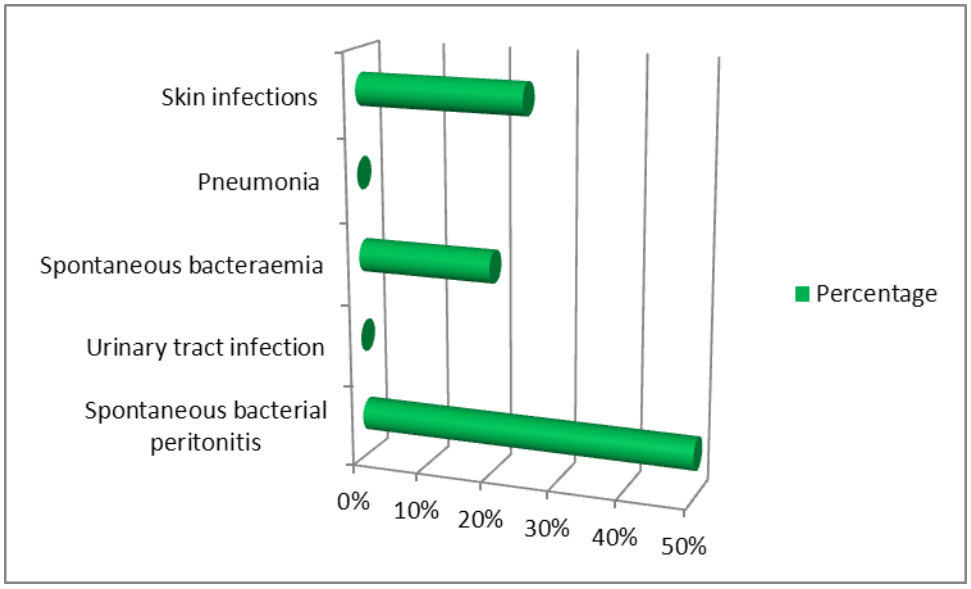


TABLE 24 : INTERPRETATION OF MIC OF VANCOMYCIN FOR METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* BY MACROBROTH DILUTION METHOD

Number of MRSA ISOLATES	MIC value	Interpretation
4	≤2µg/l	Sensitive

INTERPRETATION

≤ 2µg/ml – Susceptible

4-8µg/ml – Intermediate

≥16µg/ml - Resistant.

TABLE- 25: INTERPRETATION OF MIC OF VANCOMYCIN FOR *ENTEROCOCCUS FAECALIS* FROM ASCITIC FLUID BY MACROBROTH DILUTION METHOD

Enterococcus faecalis	MIC value	Interpretation
1	64µg/ml	Resistant

INTERPRETATION

≤ 4µg/ml – Susceptible

8-16µg/ml – Intermediate

≥32µg/ml - Resistant

TABLE-26: ANTIBIOTIC RESISTANT PATTERN IN DIFFERENT TYPES OF INFECTIONS IN DCLD PATIENTS

Type of infections in DCLD (n=81)	ESBL (n=25)		MRSA (n=4)		VRE (n=1)		MBL (n=1)	
	no	%	no	%	no	%	no	%
Spontaneous bacterial peritonitis (n= 22)	4	18	2	9	1	4.5	-	-
urinary tract infections (n=21)	11	52	-	-	-	-	-	-
Spontaneous bacteraemia (n=15)	2	13	1	7	-	-	-	-
Pneumonia(n=13)	5	38	-	-	-	-	1	8
Skin & soft tissues infections (n=10)	3	30	1	10	-	-	-	-

In 81 culture-positive infections, 31 drug resistant bacterial infections were identified: 25 ESBL (81%), four Methicillin resistant *Staphylococcus aureus*(10%) one VRE (2.3%), and one MBL (2.3%). Of the culture-positive infections, these drug resistant bacterial infections occurred in 11 of 21 (52%) of the UTI cases, 7 of 22 (32%) of the SBP, 3 of 15 (20%) of the spontaneous bacteraemia cases, 6 of 13 (46%) of the pneumonia cases and 4 of 10 (40%) of the skin and soft tissue infection cases.

CHART-14: ANTIBIOTIC RESISTANT PATTERN IN DIFFERENT TYPES OF INFECTIONS IN DCLD PATIENTS

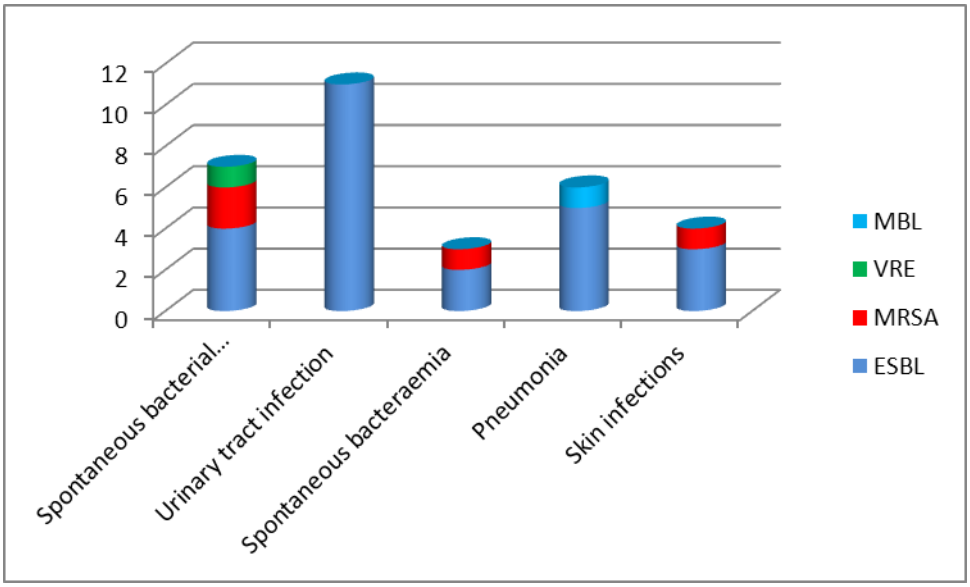


TABLE- 27: SEROLOGICAL CORRELATION OF CAUSES OF DCLD (n=150)

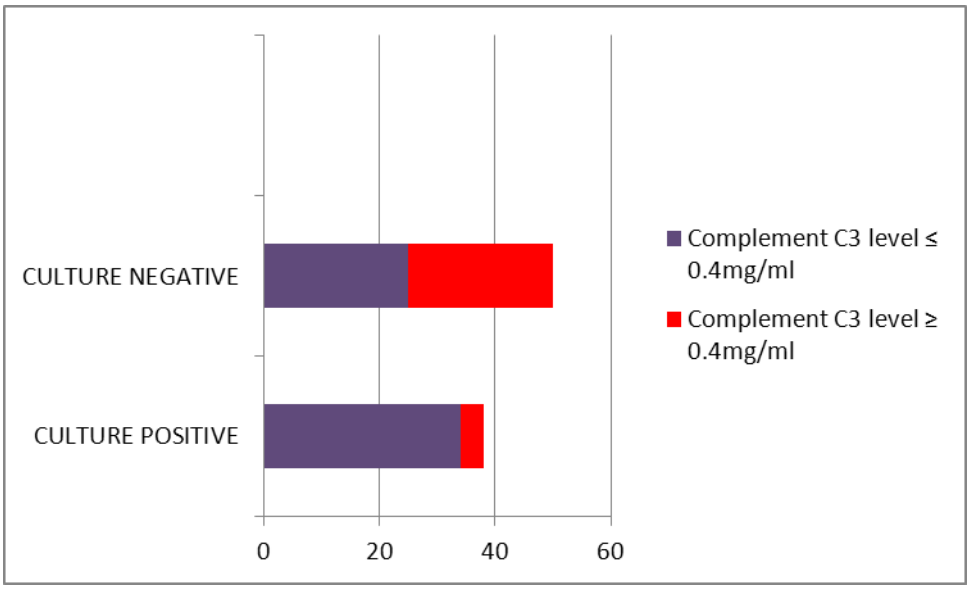
SEROLOGICAL TESTS DONE	TEST POSITIVES	Percentage
HBsAg ELISA	12	8
Anti HCV ELISA	11	7.3
Total	23/150	15.3%

Of the serological tests, Hepatitis B surface antigen was detected in 8% of the patients included in the study. Anti hepatitis C virus was found to be positive in 7.3% of the patients included in the study.

TABLE-28: DETECTION OF COMPLEMENT COMPONENT C3 CONCENTRATION BY ELISA

NO. OF SAMPLE	Total sample (n=88)	CULTURE RESULT	
		POSITIVE	NEGATIVE
Value less than 0.4mg/ml	59(67%)	35 (59%)	24 (41%)
Value more than0.4mg/ml	29(33%)	4 (14%)	25 (86%)

CHART-15: DETECTION OF COMPLEMENT COMPONENT C3 CONCENTRATION BY ELISA



- Total samples of 150, Complement C3 ELISA were done for randomly selected 88 samples with one kit due to economic constrains.

- Out of 88 patients, 67% patients were having low complement C3 level. Of which 59% patients were culture positive and 41% were culture negative.

- 33% had normal complement C3 level, of which.86% were culture negative and 14% were culture positive.

- (P value = 0.001 – Highly significant)

DISCUSSION

This study was conducted in the Institute of Microbiology, Madras Medical College, in association with other departments (Internal Medicine, Gastroenterology, and Hepatology etc), at Rajiv Gandhi Government General Hospital, Chennai. Total number of 150 DCLD patients with signs and symptoms of bacterial infections (who satisfied the inclusion criteria) were included in this study.

Among 150 DCLD patients, males 145 (97%) were predominant group when compared to females 5(3%).(TABLE-1). This predilection of higher frequency rates among male is attributed towards the presence of underlying risk factors like alcoholism [134]. Mathurin S et al [13] 2009 has reported that DCLD incidence rates are more frequently observed in males (94.8%) when compared with females at all age groups.

The common age groups, belonged to 41-50years (33.33%), and followed by 31-40 years (29.33%), 51-60years (26%) of age. Less than 30years were less commonly involved in this study (TABLE-2,5). These 41-50years age group patients with DCLD had low complement level and also had low phagocytic activity in serum and ascitic fluid, which predisposed to bacterial infections. Basra S et al 2011[131] has reported that the majority of cirrhosis occurred before the age of 60years and Hoefs 2002 et al [132] has reported that the similar age group 41-60 years, more commonly involved in bacterial infections in DCLD patients.

In our study, the main causes of decompensated liver disease was alcoholic liver diseases (84%), followed by viral hepatitis (10%), both alcoholic liver diseases with viral hepatitis (5.34%) and Cryptogenic –cause unknown (0.66%) (TABLE - 4). Acetaldehyde is a metabolic product of alcohol which is excreted by liver and it may interact with proteins and membrane lipids, causing alterations in their structure and function of liver, which may lead to cell injury and cell death and causes cirrhosis. Since Hepatitis C virus (HCV) itself is not cytopathic, liver damage in chronic hepatitis C is commonly leads to immune- mediated mechanism if the same patient consumed heavy alcohol which will accelerates this progression of liver damage into cirrhosis. Alcohol and hepatitis C virus (HCV) act synergistically to increases the incidence of cirrhosis. Mathurin S et al [13] 2009 has reported that the alcoholic etiology for DCLD was 95.4%. Study conducted by Singal AK et al 2007[26] and Ashwani K singh et al 2011[27] reported that the concomitant alcohol abuse and hepatitis C virus occur in about 14% of individuals with chronic liver disease.

Among 150 patients of DCLD, 81(54%) were culture positive (TABLE-3). In 81 culture positive isolates, 63(78%) were Gram Negative bacilli and 18 (22%) were Gram Positive cocci, which was correlated significantly [P value = 0.005] (TABLE-6). In decompensated liver disease (DCLD) patients, the most common isolates were Gram negative bacilli which may be due to translocation of normal flora (most of the normal flora in the GIT are GNB) from the gastro intestinal tract. Among bacterial infections, *Escherichia coli* were the most

common pathogen (24%). Study conducted by.Borzio M et al 2001[18] showed that the *Escherichia coli* was the most frequent pathogen (25%).

In this study, the majority of samples received and processed were ascitic fluid (39.33%), followed by urine (29.33%), sputum (18%) and wound swab (13.33%). Blood sample was collected from all the 150 (100%) patients. Among culture positive infections , spontaneous bacterial peritonitis (27%) was the most common infection due to translocation of enteric organisms from the intestine to the peritoneum and diagnostic and therapeutic paracentesis were predisposed to bacterial infections, followed by urinary tract infection (26%), spontaneous bacteraemia (19%), pneumonia (16%) and skin and soft tissues infection (12%) (TABLE - 3). Similar results were obtained in studies conducted by Puneetha Tandon et al [36] 2012, Mathurin S et al [13] 2009 and Borizia M et al [32].

In patients with spontaneous bacterial peritonitis (SBP) (27%) the most frequently isolated organisms was *E.coli* (31.82%) as it is the commonest enteric pathogen, followed by *Staphylococcus aureus* (18.18%), *Klebsiella pneumoniae* (13.63%) and *Enterococcus faecalis* (9.09%) (TABLE- 7). Similar results were obtained in studies conducted by Hoefs 2002 et al [132,] Weinstein 2000 et al [135] and· Rimland et al 2007 et al[136].Two isolates (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were isolated from both ascitic fluid and blood with same sensitivity pattern.

In this study, among 44 urine samples, 21 (26%) were culture positive. Of which *Escherichia coli* (38.09%) were the most common isolates followed by *Klebsiella oxytoca* (28.57%), *Klebsiella pneumoniae* (14.28%), *Acinetobacter baumanii* (9.52%) and *Enterococcus faecalis* (4.76%) (TABLE - 8). The incidence of urinary tract infection (UTI) is higher in decompensated cirrhotic patients with indwelling urinary catheters. Maria Pleguezuelo et al [11] 2013 has reported that the most frequent bacteria causing urinary tract infections (UTI) in DCLD patients were *E. coli* and *Klebsiella spp.*

Among 150 blood samples, 15 (19%) samples were culture positive. Of which *Klebsiella pneumoniae* (26.33%) was the most common isolate followed by *Staphylococcus aureus* (20.00%), *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus viridians* (TABLE-9). The Porto systemic shunt circulation in DCLD patients will favour the organisms to escape from phagocytosis by hepatic reticuloendothelial system, there by establishing systemic bacteraemia. Out of 15 blood culture positive samples, 2 (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) isolates were isolated from ascitic fluid also. Rooby Erachamveettil Hamza et al 2014 [137] has reported that the spontaneous bacteraemia is mainly caused by Gram negative bacilli followed by Gram positive cocci.

In the sputum samples, 13(16%) samples were culture positive. *Klebsiella oxytoca* (46.14%) was the most common isolates followed by *Klebsiella pneumoniae* (23.07%) *Pseudomonas aeruginosa* (23.07%) and *Escherichia coli* (7.69%) (TABLE-10). Some procedures like tracheal

intubation, oesophageal tamponade, and clinical conditions such as hepatic encephalopathy, and alcoholism were clearly predisposing factors for pneumonia in cirrhotic patients.Study conducted by Stefano Fagiuolia et al [143] 2013 reported that the prevalence of pneumonia in DCLD patients was 15% .

Out of 18 wound swabs, 10(12%) were culture positive. *Proteus vulgaris* (30%) was the main isolate followed by *Staphylococcus aureus*(20%), *Staphylococcus epidermidis* (20%), *Escherichia coli* and *Klebsiella pneumoniae* (TABLE-11). Lymphangitis of the lower extremities and abdominal wall are frequent in cirrhotic patients with edema or ascites which will leads to skin and soft tissue infections in DCLD patients. Study conducted by Rooby Erachamveettil Hamza et al 2014 [137] and Mohan et al 2011[144] reported that the prevalence of skin infections in cirrhosis was 10.5-12.5% and the major etiological organisms were *Staphylococcus aureus* and gram negative bacilli. This is in my study which showed *Proteus vulgaris* (30%) as the main isolate.

In the present study, all the GNB isolated from spontaneous bacterial peritonitis were100% sensitive to imipenem and most of the isolates were 75% sensitive to amino glycosides and fluoroquinolones. Out of 16 GNB, 56% only showed sensitive to third generation cephalosporins. *Pseudomonas aeruginosa* isolate showed 100% sensitivity to ceftazidime. *Acinetobacter baumanii* showed 100% sensitive to all the drugs used against for GNB (TABLE-12). In GPC, *Staphylococcus aureus* showed 50% sensitive to methicillin and 100%

sensitive to vancomycin. *Enterococcus faecalis* were 50% sensitive to vancomycin and 100% sensitive to all other drugs (TABLE-13).

Among urinary tract infections, all the GNB were 30% sensitive to third generation cephalosporins. *Klebsiella pneumoniae* was 100% sensitive to amino glycosides, fluoroquinolones carbapenem and piperacillin / Tazobactum. *Enterobacter cloacae* were 100% sensitive to all other drugs (TABLE-14). *Enterococcus faecalis* was 100% sensitive to vancomycin (TABLE-15).

In our study, all the GNB isolated from blood showed 100% sensitive to amino glycosides, carbapenem and piperacillin / Tazobactum. Out of 8 GNB in blood culture, 2 isolates (*Klebsiella pneumoniae and Pseudomonas aeruginosa*) were isolated from ascitic fluid with same sensitivity pattern (TABLE-16). *Streptococcus viridians* showed ciprofloxacin, ampicillin, cotrimoxazole and erythromycin. Out of 5 *Staphylococcus spp*, 3isolates were *Staphylococcus aureus* and 2 isolates were *Staphylococcus epidermidis*. All the *Staphylococcus aureus* and *Staphylococcus epidermidis* showed 100% sensitive to vancomycin by minimum inhibitory concentration method. *Staphylococcus aureus* were showed 67% sensitive to methicillin. All the Gram positive cocci (GPC) were 100% sensitive to fluoroquinolones (TABLE-17).

In the sputum samples, among GNB, 31% of isolates were sensitive to third generation cephalosporins. All the GNB were 100% sensitive to carbapenem except one *Klebsiella oxytoca* which was resistant to carbapenem (TABLE-18). No Gram positive cocci was isolated from sputum samples

In skin infections, among Gram Negative bacilli (GNB), 17 % were sensitive to third generation cephalosporin. *Pseudomonas aeruginosa* was sensitive to all the drugs. *E.coli* was 100% sensitive to amino glycosides, piperacillin / tazobactum, Imipenem and gentamicin. All the Gram Negative bacilli (GNB) were 100% piperacillin / tazobactum, imipenem and tetracycline (TABLE-19). 50% of Staphylococcus aureus were sensitive to methicillin and 50% of Staphylococcus aureus were resistant to methicillin. All the GPC were 100% sensitive to vancomycin by minimum inhibitory concentration method (TABLE-20).

Among 63 (78%) were Gram Negative bacilli, 25of 63 (43%) were ESBL producer. Of which, majority of ESBL isolates 11 (55%) were from urinary tract infections followed by 3 (50%) from skin & soft tissues infections, 5 (38%) from pneumonia, 4 (25%) from spontaneous bacterial peritonitis and 2 (25%) from spontaneous bacteraemia (TABLE- 21). One MBL producing *Klebsiella oxytoca* was isolated from the sputum sample (TABLE-22). Javier Fernandez et al 2013 has reported that the increased prevalence of infections caused by multiresistant bacteria in cirrhosis of which extended-spectrum β-lactamase–producing Enterobacteriaceae are more common.

Total of 13 Gram positive cocci (GPC), 9 (69%) were Methicillin sensitive and 4(31%) were Methicillin resistant, of which 2 (50%) MRSA isolates were from spontaneous bacterial peritonitis, 1(25%) MRSA isolated from spontaneous bacteraemia and 1(25%) MRSA isolate from Skin & soft tissues infection (TABLE-23, 24).

In our study, three (3) *Enterococcus faecalis* were isolated. Of which two *Enterococcus faecalis* were isolated from ascitic fluid and one *Enterococcus faecalis* was isolated from urine sample. Among the ascitic fluid isolates, One *Enterococcus faecalis* was resistant vancomycin (VRE) by minimum inhibitory concentration method (**TABLE 2**5).

In 81 culture-positive isolates, 31 were drug resistant bacterial infections were identified: 81% (25of 31) were ESBL , 13% (4/31) were Methicillin resistant Staphylococcus aureus , 3% (1/31) were vancomycin resistant Enterococci (VRE) , and 3% (1/31) were MBL (3%) (TABLE-26). Of the culture-positive isolates, these drug resistant bacterial infections occurred in 11 of 21 (52%) of the urinary tract infections (UTIs), 7 of 22 (32%) of the spontaneous bacterial peritonitis (SBP), 3 of 15 (20%) of the spontaneous bacteraemia cases, 6 of 13 (46%) of the pneumonia and 4 of 10 (40%) of the skin and soft tissue infection cases. Early and unnecessary use of higher antibiotics like third generation cephalosporins, leads to increase in the occurrence of new resistant strains. The same results obtained by Puneeta Tandon et al 2012 [145] and Fernandez J et al 2012 [146]

In our study, the prevalence rates of Hepatitis B surface antigen and Anti hepatitis C virus (HCV) by serological methods (ELISA) were found to be 8% and 7.3% respectively (TABLE 27). India is at the intermediate endemic level of hepatitis B (2-7%), with hepatitis B surface antigen (HBsAg) prevalence between 2% to 10% among the populations studied.

Out of 150 total samples, Complement component C3 ELISA was done for randomly selected 88 samples with one kit due to economic constrains. Out of 88 patients, 59(67%) patients had low complement component C3 concentration level. Of which 35(59%) patients were culture positive and 24(41%) were culture negative. Among 29(33%) DCLD patients with normal complement component C3 concentration level, 25(86%) were culture negative and 4(14%) were culture positive (TABLE 28). Complement component C3 concentration level in serum were decreased in decompensated cirrhotic patients with infections compared with decompensated cirrhotic patients without infections . Concentrations of complement component C3 level significantly correlated (p=0.001) with decompensated liver disease (DCLD) patients with infections. Many studies conducted by Mustafa G et al 2007 [80], Alper, C.A et al [81] and Colten H.R et al 1972[82] reported that the complement is mainly synthesized by hepatocytes of liver. Due to cirrhosis, all the hepatocytes are destroyed and unable to synthesize complement component C3. This reduced level of complement C3 is the one of the risk factors for bacterial infections in DCLD patients [139, 141].

SUMMARY

- Around 150 DCLD patients, who fulfilled the inclusion criteria, were included in this study.
- Males were most commonly affected with decompensated liver disease (DCLD) than female
- The vast majority of age group included in this study was 41-60 years.
- The commonest age group which showed most of the bacterial infections was between 41-50 age groups.
- .The main causes of decompensated liver disease (DCLD) was alcoholic liver diseases (84%), followed by viral hepatitis (10%), both alcoholic liver diseases and viral hepatitis (5.34%) and Cryptogenic causes(0.66%)
- In our study, the main sample received and processed was ascitic fluid (39.33%), followed by urine (29.33%), sputum (18%) and wound swab (13.33%). Blood sample was collected from all the 150 (100%) patients
- The prevalence of culture positivity according to the present study was 54% (81 / 150).
- In 81 culture positive isolates, 63(78%) were Gram Negative bacilli and 18 (22%) were Gram Positive cocci, which was correlated significantly [P value = 0.005]

- The prevalence of spontaneous bacterial peritonitis was 27%, next prevalent bacterial infections in decompensated liver disease (DCLD) patients were urinary tract infection (26%), spontaneous bacteraemia (19%) pneumonia (16%) and skin and soft tissues infection (12%).

- *Escherichia coli* (31.82%), was the most common isolates among the patients with spontaneous bacterial peritonitis (SBP) followed by *Staphylococcus aureus* (18.18%), *Klebsiella pneumoniae* (13.63%) and *Enterococcus faecalis* (9.09%)

- Similarly, in urinary tract infections, *Escherichia coli* (38.09%) was the common isolate followed by *Klebsiella oxytoca* (28.57%), *Klebsiella pneumoniae* (14.28%), *Acinetobacter baumanii* (9.52%) and *Enterococcus faecalis* (9.52%).

- *Klebsiella pneumoniae* (26.33%) was the most common isolate among decompensated liver disease (DCLD) patients with spontaneous bacteraemia followed by *Staphylococcus aureus (20.00%), Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Streptococcus viridians.*

- In pneumonia, *Klebsiella oxytoca* (46.14%) was the most common isolates followed by *Klebsiella pneumoniae* (23.07%) *Pseudomonas aeruginosa* (23.07%) and *Escherichia coli* (7.69%). No Gram positive cocci (GPC) was isolated from sputum samples.

- Most of the organisms were sensitive to amino glycosides and 50% sensitive to fluoroquinolones.

- All the GNB were 100% sensitive to carbapenem except for one isolate of *Klebsiella oxytoca* from sputum sample.

- Among 63 (78%) Gram Negative bacilli, 25 (43%) were ESBL producer. Of which, majority of ESBL isolates 55% (11/20) were from urinary tract infections followed by 50% (3/6) from skin & soft tissues infections, 38% (5/13) from pneumonia, 25% (4/16) from spontaneous bacterial peritonitis and 25% (2/8) from spontaneous bacteraemia. One MBL producing *Klebsiella oxytoca* was isolated from the sputum sample .

- Total of 13 Gram positive cocci (*Staphylococcus spp*), 9 (69%) were Methicillin sensitive and 4(31%) were Methicillin resistant, of which 50% of MRSA isolated from spontaneous bacterial peritonitis , 25% from spontaneous bacteraemia and 25% from Skin infections.

- Out of 13 Staphylococcus spp, only four [31%] isolates were methicillin resistant *Staphylococcus aureus* (MRSA) and they were found to be sensitive to vancomycin by minimum inhibitory concentration (MIC) by Macro broth dilution method.

- In our study, three (3) *Enterococcus faecalis* were isolated. Of which two *Enterococcus faecalis* were isolated from ascitic fluid and one

Enterococcus faecalis was isolated from urine sample. One vancomycin resistant Enterococci (VRE) isolated from ascitic fluid

- In 81 culture-positive isolates, 31 were drug resistant bacterial isolates were identified: 81% (25of 31) were ESBL , 13% (4/31) were Methicillin resistant Staphylococcus aureus , 3% (1/31) were vancomycin resistant Enterococci (VRE) , and 3% (1/31) were MBL (3%) . Of the culture-positive isolates, these drug resistant bacterial infections occurred in 11 of 21 (52%) of the urinary tract infections (UTIs), 7 of 22 (32%) of the spontaneous bacterial peritonitis (SBP), 3 of 15 (20%) of the spontaneous bacteraemia cases, 6 of 13 (46%) of the pneumonia and 4 of 10 (40%) of the skin and soft tissue infection cases.

- In our study, the prevalence of Hepatitis B surface antigen and Anti hepatitis C virus by serological methods (ELISA) were found to be 8% and 7.3% respectively.

- Out of 88 patients, 59(67%) patients had low complement component C3 level. Of which 35(59%) patients were culture positive and 24(41%) were culture negative. 29(33%) had normal complement component C3 level, of which 25(86%) were culture negative and 4(14%) were culture positive.

CONCLUSION

Bacterial infections are more frequently seen in decompensated liver disease (DCLD) patients than those with compensated liver disease patients. It is the most important cause of morbidity and mortality in decompensated liver disease (DCLD) patients

Bacterial infections in decompensated liver disease are due to invasive practical procedures, malnutrition, derangement of gut flora – intestinal stasis, bacterial over growth, increased intestinal permeability, impaired host defence mechanisms against infection. In the host defence mechanisms, impaired function of the reticuloendothelial system, deficiency of complement component level mainly C3 and impaired opsonisation activity have been implicated in the pathogenesis of the increased susceptibility to infections in patients with decompensated liver disease (DCLD).

In DCLD patients, the spontaneous bacterial peritonitis is the most common infection followed by urinary tract infections (UTI), spontaneous bacteraemia, pneumonia and skin infections.

The vast majority of infections were caused by Gram negative bacilli than Gram positive cocci. In Gram negative bacilli, the common isolate was *Escherichia coli*. In Gram positive cocci, *Staphylococcus aureus* was the common isolate. In culture positive infections, 50% drug resistant bacterial infections were identified, mainly ESBL followed by MRSA, VRE and MBL.

A definitive etiologic diagnosis with its antimicrobial susceptibility pattern is used to arrive at an appropriate antibiotic policy and to know the changing trends in the nature of the microbial agents. This will help in minimizing the emergence and spread of drug resistant pathogens to the community.

Antibiotic prophylaxis must be restricted to selected patients with high risk for the development of bacterial infections. Encouraging the use of first line antibiotics and to avoid unnecessary use of higher antibiotics like third generation cephalosporins will help to reduce the occurrence of new resistant strains.

Antibiotic de-escalation is a mechanism whereby the provision of effective initial antibiotic treatment is achieved while avoiding unnecessary antibiotic use that would promote the development of resistance strains. It is a key element within antimicrobial stewardship programs and treatment for serious bacterial infections. The embodiment of de-escalation is that based on microbiology results around the day 3 therapy point; the empiric antibiotic(s) that were started are stopped or reduced in number and/or narrowed in spectrum.

So, early identification of the source of bacterial infections in decompensated liver diseases patients and appropriate antibiotic treatment can significantly reduce hospital stay and morbidity and improve survival rate.

Figure -1: DIRECT GRAM STAINING FROM ASCITIC FLUID SHOWED PUS CELLS AND GRAM NEGATIVE BACILLI

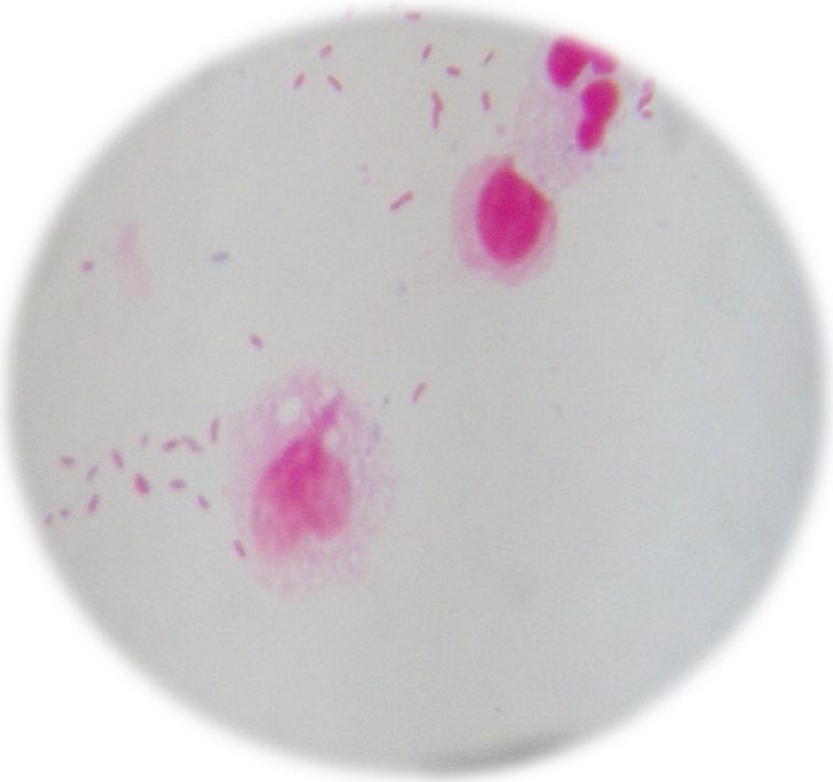


FIGURE 2:GROWTH OF *KLEBSIELLA PNEUMONIAE* ON MAC CONKEY AGAR

FIGURE 3:BIOCHEMICAL REACTIONS OF *KLEBSIELLA PNEUMONIAE*

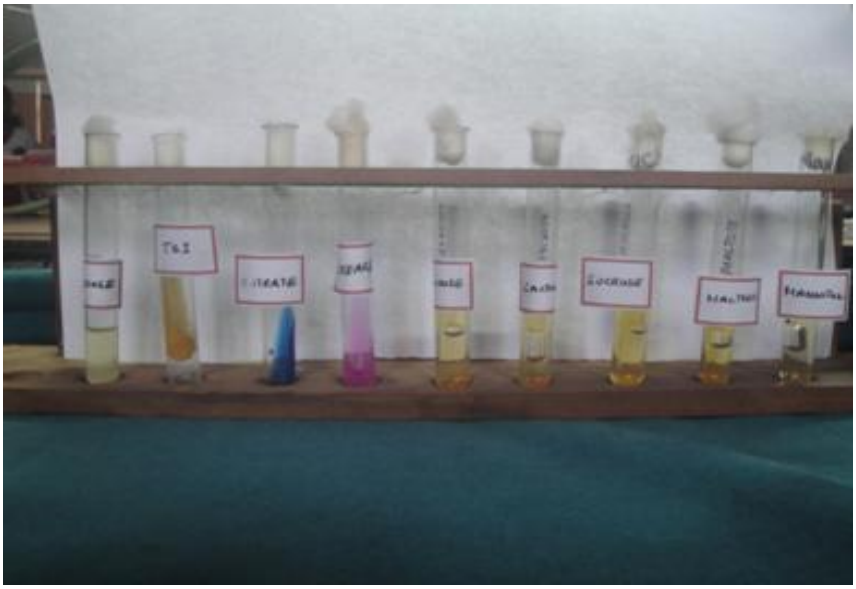


FIGURE 4: **ANTIBIOGRAM FOR *ESCHERICHIA COLI***

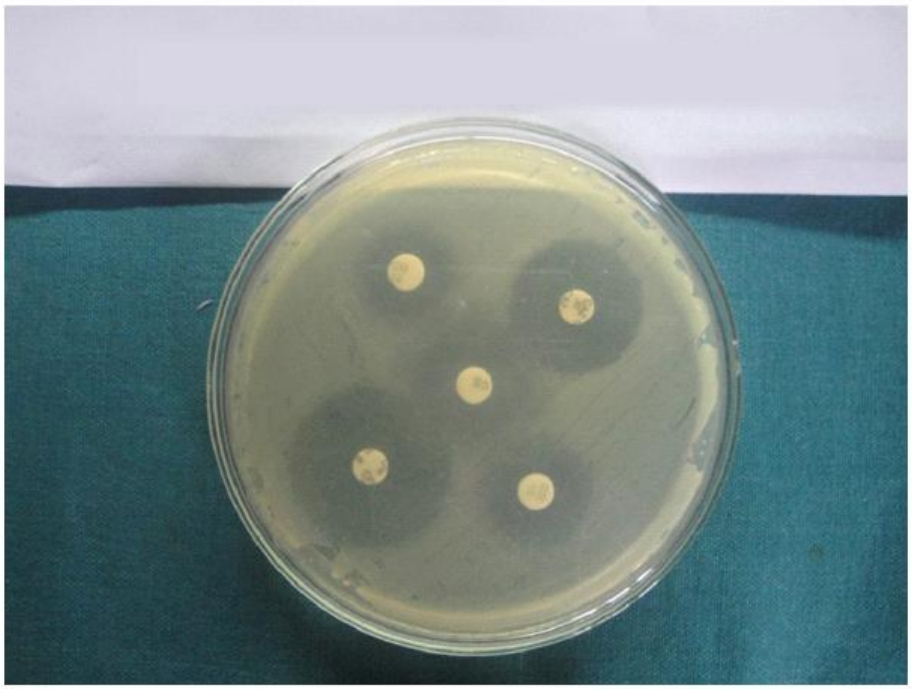


FIGURE 5:NOVOBIOCIN SENSITIVE AND POLYMYXIN RESISTANT *STAPHYLOCOCCUS EPIDERMIDIS*

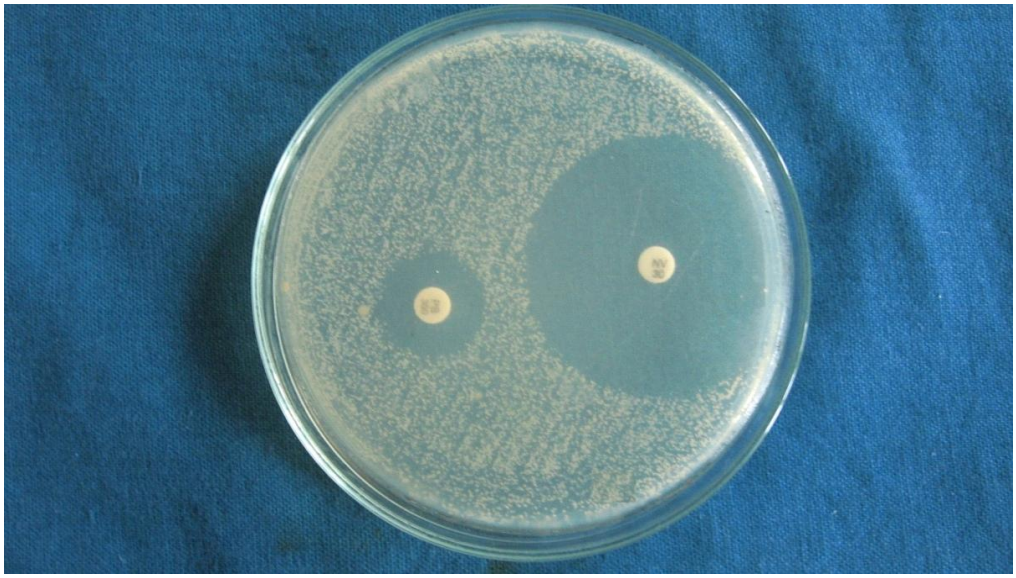


FIGURE 6:METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

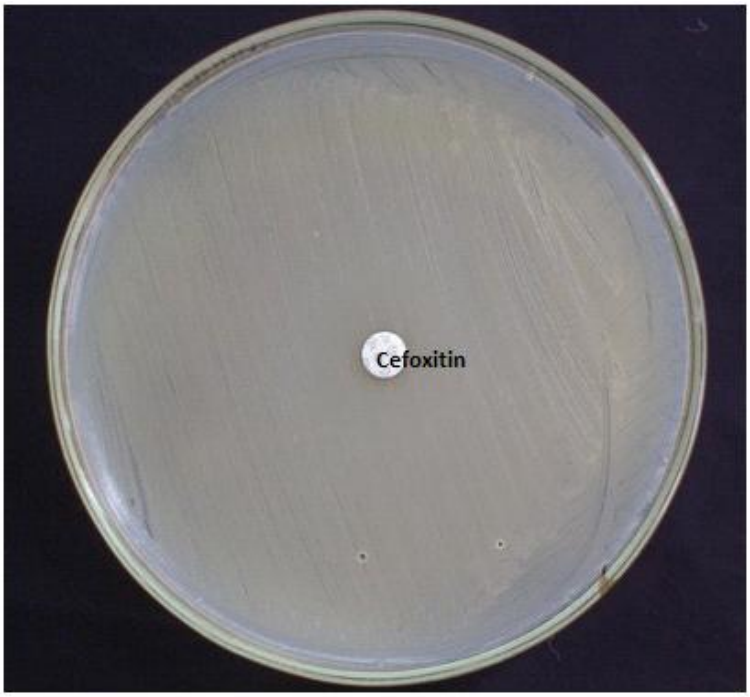


FIGURE 7:DOUBLE DISK DIFFUSION SYNERGY TEST FOR DETECTION OF ESBL

FIGURE 8: PHENOTYPIC CONFIRMATORY DOUBLE DISK TEST FOR DETECTION OF ESB

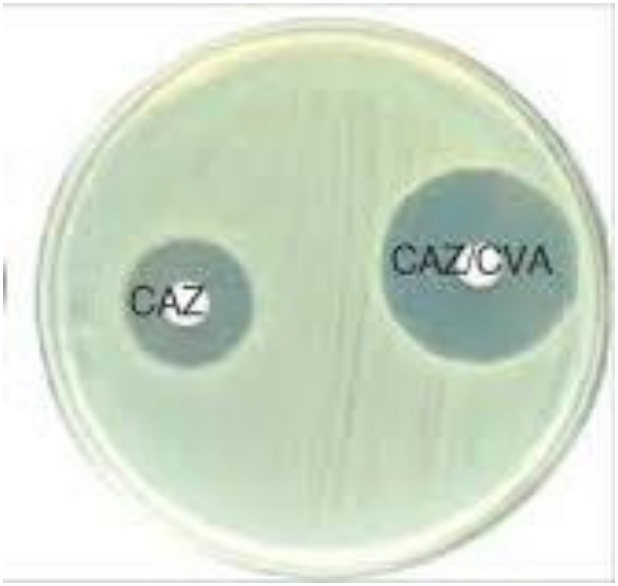


FIGURE 9:MINIMUM INHIBITORY CONCENTRATION (MIC) FOR DETECTING MEROPENEMRESISTANCE IN *KLEBSIELLA OXYTOCA- 1*6µg/ml.

FIGURE 10:MINIMUM INHIBITORY CONCENTRATION (MIC) FOR DETECTING VANCOMYCIN RESISTANCE IN *ENTEROCOCCUS FAECALIS*-64µg/ml.

FIGURE 11:DETECTION OF HBsAg BY ENZYME LINKED IMMUNOSORBENT ASSAY

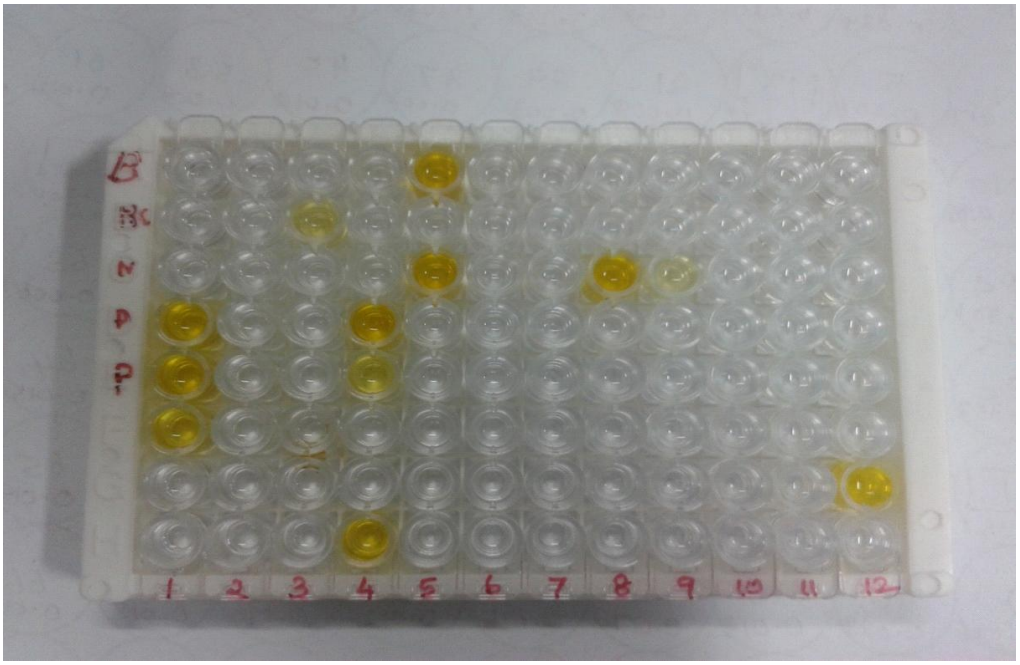
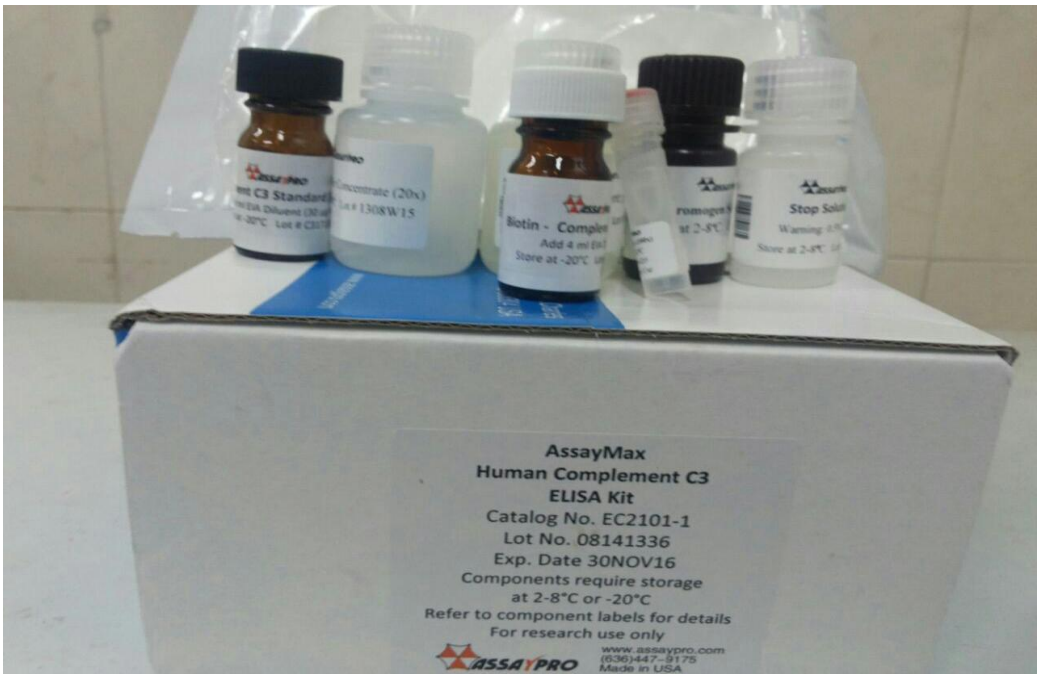


FIGURE 12:EDETECTION OF ANTI HCV BY ENZYME LINKED IMMUNOSORBENT ASSAY

FIGURE 13:HUMAN COMPLEMENT C3 ELISA KIT



APPENDIX - I

ABBREVIATIONS

ATCC	-	American Type Culture Collections
CFU	-	Colony forming unit
CLSI	-	Clinical & Laboratory Standards Institute
DCLD	-	Decompensated liver disease
DDST	-	Double Disk Diffusion Synergy Test
E.coli	-	Escherichia coli
ESBL	-	Extended Spectrum Beta Lactamases
GNB	-	Gram – Negative Bacilli
GPC	-	Gram – Positive Cocci
MBL	-	Metallo ß-Lactamases
MIC	-	Minimum Inhibitory Concentration
MRSA	-	Methicillin Resistant Staphylococcus aureus
MSSA	-	Methicillin Sensitive Staphylococcus aureus
PCDDT	-	Phenotypic Confirmatory Disk Diffusion Test
RES	-	Reticuloendothelial system
SBP	-	Spontaneus bacterial peritonitis
UTI	-	Urinary tract infection

APPENDIX II

A. STAINS AND REAGENTS

1. Gram staining

Methyl violet (2%)	l0g Methyl violet in 100ml absolute alcohol in 1 litre of distilled water (primary stain)
Grams Iodine	l0g Iodine in 20g KI (fixative)
Acetone	Decolourising agent
Carbol fuchsin 1%	Secondary stain.

B. MEDIA USED

1. Mac Conkey agar

Peptone	20g
Sodium taurocholate	5 g
Distilled Water	1 ltr
Agar	20 g
2% neutral red in 50% ethanol	3.5ml
10% lactose solution	l00ml

Dissolve peptone and taurocholate in water by heating. Add agar and dissolve it in steamer. Adjust pH to 7.5. Add lactose and neutral red shake well and mix.Heat in free steam (100°C) for 1 hour, then autoclave at 115°C for 15 minutes.

2. Nutrient agar

Peptic digest of animal tissue	5g
Sodium chloride	5g
Beef extract	1.5g
Yeast extract	1.5g
Agar	15gm

Final pH 7.4±0.2

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure (120°C) for 15 minutes.

3. Blood agar (5% sheep blood agar)

Peptone	l0g
NaCl	5g
Distilled water	1 Ltr
Agar	l0g

Dissolve ingredients in distilled water by boiling, and add 5% sheep blood(sterile) at 55°C adjust pH to 7.4.

4. Chocolate agar

Sterile defibrinated blood	10 ml
Nutrient Agar (melted)	100 ml

When the temperature was about 75°C, sterile blood was added with constant agitation. After addition of blood, kept in water bath and heating was continued till the blood changed to chocolate colour. Cooled to about 50° C and poured about 15ml into petri dishes with sterile precaution.

5. **Mueller- Hinton Agar**

Beef infusion	300ml
Caesein hydrolysate	17.5g
Starch	1.5g
Agar	l0g
Distilled water	lltr

pH = 7.4

Sterilise by autoclaving at 121°C for 20 mins

6. Robertson's Cooked Meat Broth

Fresh bullock heart	5 00g
Water	500ml
Sodium hydroxide, lmol/1	1.5ml
Liquid filtered from cooked meat	500ml
Peptone	2.5g
NaCl	1.25g

7. Thioglycollate broth

Pancreatic digest of casein	15gms
Yeast extract	5gms
Dextrose (Glucose)	5.5gms
Sodium chloride	2.5gms
L-Cystine	0.5gms

Autoclaved at 15 lbs pressure (121°C) for 20 minutes.

Note: If more than the upper one-third of the medium has acquired a pink colour, the medium may be restored once by heating in a water bath or until the pink colour disappears.

C. MEDIA REQUIRED FOR BIOCHEMICAL IDENTIFICATION

1. Oxidase Reagent

Tetra methyl p-phenylene diamine dihyrochloride- 1% aqueous solution.

2. Catalase

3% hydrogen peroxide

3. Indole test

Kovac's reagent

Amyl or isoamyl alcohol 150ml Para dimethyl amino benzaldehyde lOg Concentrated hydrochloric acid 50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator. Shake gently before use.

4. Christensen's Urease test medium

Peptone	lg
Sodium chloride	5g
Dipotassium hydrogen phosphate	2g
Phenol red	6ml
Agar	20g
Distilled water	1 ltr
10% sterile solution of glucose	10ml
Sterile 20% urea solution	100ml

Sterilize the glucose and urea solutions by filtration. Prepare the basal medium without glucose and urea, adjust to pH 6.8-6.9 and sterilize by autoclaving in a flask at 121°C for 30min. Cool to about 50°C, add the glucose & urea, and tube the medium as slopes.

5. Simmon's Citrate Medium

Koser's medium	1 ltr
Agar	20 g
Bromothymol blue 0.2%	40ml

Dispense, autoclave at 121°C for 15 min and allow to set as slopes

6. Triple Sugar Iron medium

Beef extract	3g
Yeast extract	3g
Peptone	20g
Glucose	lg
Lactose	10 g
Sucrose	l0g
Ferric citrate	0.3g
Sodium chloride	5g
S odum thiosulphate	0.3g
Agar	12g
Phenol red 0.2% solution	12ml
Distilled water	1 ltr

Heat to dissolve the solids, add the indicator solution, mix and tube. Sterilize at 121°C for 15 min and cool to form slopes with deep butts.

7. Glucose phosphate broth

Peptone	5g
Dipotassium hydrogen phosphate	5g
Water	1 ltr
Glucose 10% solution	50ml

Dissolve the peptone and phosphate and adjust the pH to 7.6. Filter dispense in 5ml amounts and sterilize at 121°C for 15min. Sterilize the glucose solution by filtration and add 0.25ml to each tube.

Methyl Red Reagent

Methyl Red	l0mg
Ethyl alcohol	30ml
Distilled water	20ml

Voges Proskauer Reagent

Reagent A: Alpha naphthol	5g
Ethyl alcohol	100ml
Reagent B: Potassium hydroxide	40g
Distilled water	100ml

8. Peptone water fermentation test medium

To the basal medium of peptone water, add sterilised sugars of 1% indicator bromothymol blue with Durham's tube. Basal medium peptone water Sugar solutions:

Sugar	1ml
Dislilled water	100ml

pH = 7.6.

9. Mannitol motility medium

Agar	5g
Peptone	lg
Potassium nitrate	1g
Mannitol	2g
Phenol red indicator	
Distilled water	1000ml
pH	7.2

10. Phenolphthalein diphosphate agar

- Sterilize a 1% aqueous solution of sodium phenolphthalein diphosphate by filtration and store at 4°C
- Add 10ml of this solution to 1000ml melted nutrient agar cooled to 50°C and pour plates
- Grow the staphylococcus overnight at 37°C on the medium
- Invert the plate and pour a few drops of ammonia solution SG 0.88 into the lid
- Read as positive a culture whose colonies turn bright pink within a few minutes. The colour soon fades.

11. Potassium nitrate broth

Potassium nitrate (KNO3)	0.2gm
Peptone	5.0gm
Distilled water	100ml

The above ingredients were mixed and transferred into tubes in 5 ml amount and autoclaved.

12. Phenyl alanine deaminase test

Yeast Extract	3g
Dl-Phenylalamine	2 g
Disodium hydrogen phosphate	l g
Sodium Chloride	5 g
Agar	12g
Distilled water	1 lr
PH	7.4

Distributed in tubes and sterilized by autoclaving at 121° C for 1 5 minutes, allowed to solidify as long slopes.

13. Sugar fermentation medium

Peptone	15g
Andrade's indicator	10 ml
Sugar to be tested	20g
Water	1 litre

Andrade's indicator is prepared from 0.5% aqueous acid fuchsin to which sufficient 1M sodium hydroxide has been added to turn the colour of the solution yellow.

Dissolve the peptone and Andrade's indicator in 1 litre of water and add 20g of the sugar; sugars to be tested generally include glucose, sucrose, lactose and maltose. Distribute 3ml amounts in standard test tubes containing an inverted Durham tube. Sterilize by steaming at 100 degree C for 30 min on 3 consecutive days.

ANNAXURE - I

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To

Dr. J.RAJESWARI,
Post Graduate in MD Microbiology,
Institute of Microbiology,
Madras Medical College, Chennai-3.

Dear **Dr. J.RAJESWARI,**

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"A study on Bacterial infections and their antimicrobial susceptibility pattern in Decompensated liver disease patients in a Tertiary care Hospital." "** No.18122013

The following members of Ethics Committee were present in the meeting held on 11.12.2013 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2. Prof. B. Kalaiselvi, MD Vice Principal, MMC, Ch-3	-- Member Secretary
3. Prof. Ramadevi, Director i/c, Instt. of Biochemistry, Chennai.	-- Member
4. Prof. P. Karkuzhali, MD for Dr. V. Ramamoorthy Prof. Instt. of Pathology, MMC, Ch-3	-- Member
5. Thiru. S. Govindasamy, BABL	-- Lawyer
6. Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

20/12/13

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE-II

PROFORMA

- Name : \IP no:
- Age: Sex: Ward:
- Occupation:
- Address:
- Presenting complaints:
    - Abdominal swelling
    - jaundice
    - High grade fever
    - Altered sensorium
    - Cough with expectoration
    - Burning micturition
    - Hemoptysis:
    - Abdominal pain:
    - abdominal discomfort
    - Leg swelling

Past history:

- Hepatitis
- Known tuberculosis patient

- Chronic steroid intake
- Diabetes mellitus
- Malignancy
- Chemotherapy
- Transplant recipient
- HIV

Personal history:

- Alcohol intake:
- Cigarette smoking:
- Exposure to chemicals occupationally

Physical and General Examination :

BP : PR :

CVS : RS :

P/A : P/R :

Provisional Diagnosis:

Laboratory Investigations :

1. Complete haemogram .
2. Liver function test
3. Renal function test

Serological investigations

- HbsAg
- ANTI-HCV
- HIV

Other Investigations

Ultrasono gram:

Chest X ray:

CT Abdomen :

MICROBIOLOGICAL INVESTIGATION:

Sample collected:

1.Ascitic fluid.

2. serum.

3. Blood sample.

4.urine.

5.sputum.

6. Wound swab

Direct examination:

Gram’s stain:

Bacterial Culture (other samples) :

- MAC
- BAP
- CAP

Blood culture:

- Inoculated onto BHI broth with subculture onto
  - MAC
  - BAP
  - CAP

Isolate identified in the sample:

Antibacterial susceptibility pattern :

Complement component C3 level:

ANNEXURE –III

CONSENT FORM

STUDY TITLE:

A Study on bacterial infections and their antimicrobial susceptibility pattern in Decompensated liver disease patients (DCLD) in a tertiary care hospital

I……, hereby give consent to participate in the study conducted by **Dr.J.RAJESWARI**, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my **ascitic fluid / urine/ sputum/ wound swab/ blood** for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature / Thumb impression of the patient / relative

Place :
Date :

Patient Name & Address:

Signature of the investigator

S. No	Patient's Age	Sex	Diagnosis	Causes of cirrhosis			Sample	CULTURE RESULT		Complememnt C3 level [mg/ml]	Antibiotic sensitivity																							
				Alcohol	Viral	Cryptogeni		Blood	OTHER SAMPLE		Amik	Genta	Cef	CS	Cip	Ofl	Imi	Pip/Taz	Chlor	Tetra	Cot	Clin	Ery	Pen	Ceph	Van	NOR	Nitr	Amp	Amox	Cefoxitin	HLG
1	46	M	DCLD	y			Ascitic fluid,Blood	Klebsiella pneumoniae	Klebsiella pneumoniae	0.42	S	S	S	S	R	S	S	S														
2	35	M	DCLD	y			Ascitic fluid,Blood	No growth	E.Coli (ESBL)	0.16	S	R	R	S	S		S	S														
3	50	M	DCLD	y			Blood, Sputum	Klebsiella pneumoniae	Normal throat commensal	1.92	S	S	S	S	S		S	S			S											
4	31	M	DCLD		Y		URINE,Blood	No growth	Enterobacter cloaca	1.6	S	S	S	S	S	S	S	S														
5	30	M	DCLD		Y		Blood, Ascitic fluid	No growth	No growth	0.44																							
6	47	M	DCLD	Y			Sputum, Blood	No growth	E.Coli (ESBL)	0.2	S	R	R	S		R	S	S														
7	34	M	DCLD		Y		Ascitic fluid,Blood	No growth	No growth	0.4																							
8	67	M	DCLD	Y			Ascitic fluid,Blood	No growth	E.Coli (ESBL)	0.21	S	S	R	S	S		S	S														
9	40	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.136																							
10	45	M	DCLD		Y		Wound swab,Blood	No growth	Proteus vulgaris (ESBL)	0.206	S	S	R	S		R	S	S			S											
11	38	M	DCLD		Y		Ascitic fluid,Blood	No growth	No growth	0.256																							
12	54	M	DCLD	Y			Ascitic fluid,Blood	No growth	Proteus mirabilis	0.16	S	S	S	S	S			S														
13	54	M	DCLD	Y			URINE,Blood	No growth	E.Coli	0.24	S	S	S	S	S	S	S	S														
14	34	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.56																							
15	38	M	DCLD	Y			URINE,Blood	No growth	No growth	0.64																							
16	42	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca (ESBL)	0.4	S	S	R	S	R		S	S									S	R				
17	48	M	DCLD	Y			Sputum, Blood	No growth	Normal flora grown in culture	0.32																							
18	45	M	DCLD	y	Y		Ascitic fluid,Blood	No growth	Acinetobacter	0.16	S	S	S	S	S		S	S														
19	36	M	DCLD	y			Sputum, Blood	No growth	Klebsiella oxytoca (ESBL)	0.24	S	S	R	S	R		S	S			S											
20	47	M	DCLD		Y		URINE,Blood	No growth	No growth	0.44																							
21	49	M	DCLD	y			Blood, Ascitic fluid	Streptococcal viridans	No growth	0.32	S				S				S	S	S		R						S				
22	42	M	DCLD	y			Blood, Urine	Stapylococcus aureus (MRSA)	No growth	0.4	S	S			R					R	R		R	R					S		R		
23	45	M	DCLD	y			Blood, Sputum	No growth	Normal throat commensal	2																							
24	52	M	DCLD	y			Ascitic fluid,Blood	No growth	No growth	0.28																							
25	60	M	DCLD	y			Wound swab,Blood	No growth	No growth	0.72																							
26	35	M	DCLD		Y		Ascitic fluid,Blood	No growth	E.Coli	0.32	S	S	R	R	R		S	S														
27	40	M	DCLD	y			URINE,Blood	No growth	No growth	0.46																							
28	30	M	DCLD	y			Sputum, Blood	No growth	Klebsiella oxytoca	0.32	R	R	R	R	R		R	R			R											
29	58	M	DCLD	y			Ascitic fluid,Blood	No growth	No growth	0.48																							
30	44	M	DCLD	y			URINE,Blood	No growth	Candida non albicans	0.38																							
31	40	M	DCLD	y			Wound swab,Blood	No growth	Proteus vulgaris	0.24	R	R	R	S		S	S	S			S											
32	40	M	DCLD	y			Blood, Ascitic fluid	No growth	No growth	0.28																							
33	70	F	DCLD		y		Wound swab,Blood	No growth	Staphylococcus epidermidis(MS)	0.256	S	S			R					S	S		S	S					S		S		
34	57	M	DCLD	y			Wound swab,Blood	No growth	E.Coli (ESBL)	0.48	S	S	R	S		R	S	S														
35	43	M	DCLD		y		Blood, Urine	Staphylococcus epidermidis (MSSA)	No growth	0.45	S	S			S					R	S		S	S					R		S		
36	50	F	DCLD		Y		Ascitic fluid,Blood	No growth	No growth	1.4																							
37	55	M	DCLD	y			Blood, Sputum	No growth	Normal throat commensal	0.24																							
38	35	M	DCLD		Y		Ascitic fluid,Blood	No growth	No growth	2																							
39	42	M	DCLD	y			URINE,Blood	No growth	Klebsiella pneumoniae	0.38	S	S	S	S	S		S	S									R	S				
40	35	M	DCLD	y			Ascitic fluid,Blood	No growth	No growth	0.54																							
41	56	M	DCLD	y			Blood, Urine	No growth	No growth	0.32																							

S. No	Patient's Age	Sex	Diagnosis	Causes of cirrhosis			Sample	CULTURE RESULT		Complememnt C3 level [mg/ml]	Antibiotic sensitivity																					
				Alcohol	Viral	Cryptogeni		Blood	OTHER SAMPLE		Amik	Genta	Cef	CS	Cip	Ofl	Imi	Pip/Taz	Chlor	Tetra	Cot	Clin	Ery	Pen	Ceph	Van	NOR	Nitr	Amp	Amox	Cefoxitin	HLG
42	43	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.52																						
43	40	M	DCLD	Y			URINE,Blood	No growth	E.Coli	0.2	S	S	S	S	S		S	S									S	R				
44	62	M	DCLD	Y			URINE,Blood	No growth	E.Coli (ESBL)	0.32	S	S	R	S	S		S	R									R	R				
45	39	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.48																						
46	50	M	DCLD	Y			URINE,Blood	No growth	Klebsiella pneumoniae (ESBL)	0.24	S	S	R	S	S		S	S									R	S				
47	48	M	DCLD	y			Sputum, Blood	No growth	Pseudomonas aeruginosa	0.38	S	S	S	S	S		S	S			R											
48	42	M	DCLD	Y			URINE,Blood	No growth	Klebsiella pneumoniae (ESBL)	0.38	S	S	R	S	S		S	S									S	R				
49	50	M	DCLD	Y			URINE,Blood	No growth	E.Coli	0.04	S	R	S	S	S		S	S									R	R				
50	40	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca (ESBL)	0.36	S	S	R	S	S		S	S									R	R				
51	46	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella pneumoniae	0.52	S	S	S	S		S	S	S			S											
52	35	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth	0.47																						
53	58	M	DCLD	Y	Y		Ascitic fluid,Blood	No growth	Citrobacter koseri	0.15	S	R	S	S	S																	
54	43	M	DCLD	Y			URINE,Blood	No growth	Candida non albicans	1.08																						
55	51	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.65																						
56	58	M	DCLD	Y			Ascitic fluid,Blood	No growth	Klebsiella pneumoniae	0.37	R	R	R	R	S		S	S	R	R												
57	50	M	DCLD	Y			Blood, Ascitic fluid	Klebsiella pneumoniae	No growth	0.24	s	S	S	S	S		S	S		S	S											
58	35	M	DCLD	Y			Blood, Sputum	No growth	Normal throat commensal	0.25																						
59	35	M	DCLD	Y			Blood, Wound swab	No growth	No growth	0.52																						
60	37	M	DCLD	y			Blood, Urine	Klebsiella pneumoniae (ESBL)	No growth	0.18	S	R	R	S	R		S	S		S	R											
61	55	M	DCLD	y			URINE,Blood	No growth	No growth	0.46																						
62	30	M	DCLD		Y		Ascitic fluid,Blood	No growth	No growth	0.25																						
63	46	M	DCLD	y			Wound swab,Blood	No growth	No growth	0.52																						
64	44	M	DCLD	y			Blood, Ascitic fluid	Klebsiella pneumoniae	No growth	0.32	R	R	R	R		R	S	S	R	R	S											
65	50	M	DCLD	y			Blood, Wound swab	Stapylococcus aureus (MRSA)	No growth	0.14	S	S			R					S	S		R	R					R		R	
66	42	M	DCLD	Y			Blood, Sputum	No growth	Normal throat commensal	0.4																						
67	57	M	DCLD	y			Blood, Urine	Pseudomonas aeruginosa	No growth	1	S	S	S	S		S	S	S														
68	59	M	DCLD	y			Sputum, Blood	No growth	Klebsiella pneumoniae (ESBL)	0.24	R	R	R	S		R	S	S			R											
69	60	F	DCLD		y		Ascitic fluid,Blood	No growth	Enterococcus faecalis	0.32									S	R			R	S		S			S			R
70	42	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca (ESBL)	0.2	R	R	R	S	R		S	S									R	R				
71	42	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.12																						
72	46	M	DCLD	Y			URINE,Blood	No growth	E.Coli (ESBL)	0.38	S	R	R	S	S		S	S									S	R				
73	59	M	DCLD	Y			Ascitic fluid,Blood	No growth	E.Coli	0.28	S	S	S	S	S		S	S														
74	55	M	DCLD	Y			URINE,Blood	No growth	Acinetobacter baumanii (ESBL)	0.38	R	R	R	S	S		S	S									R	S				
75	53	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella pneumoniae(ESBL)	0.29	R	R	R	S		S	S	S			R											
76	40	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.33																						

S. No	Patient's Age	Sex	Diagnosis	Causes of cirrhosis			Sample	CULTURE RESULT		Complememnt C3 level [mg/ml]	Antibiotic sensitivity																					
				Alcohol	Viral	Cryptogeni		Blood	OTHER SAMPLE		Amik	Genta	Cef	CS	Cip	Ofl	Imi	Pip/Taz	Chlor	Tetra	Cot	Clin	Ery	Pen	Ceph	Van	NOR	Nitr	Amp	Amox	Cefoxitin	HLG
77	39	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella oxytoca	0.4	S	S	S	S	S		S	S			S										
78	30	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth	0.8																						
79	60	M	DCLD	Y			Ascitic fluid,Blood	No growth	Stapylococcus epidermidis(MS)	0.28	R	S			S					S	R		S	S					S		S	
80	58	M	DCLD	y			URINE,Blood	No growth	Candida non albicans	0.25																						
81	35	M	DCLD	y			Ascitic fluid,Blood	Pseudomonas aeruginosa	Pseudomonas aeruginosa	0.16	R	S	R	R	R		S	S													
82	40	M	DCLD	Y			Sputum, Blood	No growth	Norrmal throat commensal	0.34																						
83	42	M	DCLD	Y			URINE,Blood	No growth	E.Coli (ESBL)	0.34	S	R	R	S	R		S	R									S	R			
84	40	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth	0.56																						
85	30	M	DCLD	Y			Ascitic fluid,Blood	No growth	Klebsiella oxytoca	0.32	S	S	S	S	S		S	S													
86	78	M	DCLD	Y			Sputum, Blood	No growth	Normal thoat commensal grown	0.36																						
87	36	M	DCLD	Y			URINE,Blood	No growth	No growth	0.48																						
88	42	M	DCLD	Y			URINE,Blood	No growth	Enterococcus faecalis	0.26									S	S			R	R		S			R			R
89	36	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
90	42	M	DCLD	Y			Sputum, Blood	No growth	Pseudomonas aeruginosa (ESBL)		S	S	R	S	R		S	R			S										
91	40	M	DCLD	Y			Blood, Wound swab	Stapylococcus aureus (MSSA)	No growth		S	R			S					S	S		S	S					S		S	
92	37	M	DCLD	Y			Blood, Sputum	E.Coli	Normal throat commensal		S	S	S	S	S		S	S			S										
93	60	M	DCLD	Y			Blood,Urine	Staphylococcus epidermidis (MSSA)	No growth		S	R			S					R	S		S	S					S		S	
94	54	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth																							
95	43	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca (ESBL)		R	R	R	S	R		S	S									S	S			
96	40	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
97	61	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth																							
98	57	M	DCLD	Y			Blood, Wound swab	No growth	No growth																							
99	47	M	DCLD	Y			Blood, Sputum	Streptococcal viridans	Normal throat commensal		S				S				R	R	S		S						S			
100	55	M	DCLD	Y			Wound swab,Blood	No growth	Pseudomonas aeruginosa		S	S	S	S		S	S	S														
101	37	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
102	39	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella oxytoca		S	R	R	R	S		S	S			S										
103	54	M	DCLD	Y			Ascitic fluid,Blood	No growth	Stapylococcus aureus (MRSA)		S	R			S					S	R		S	R					S		R	
104	42	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																						R	
105	30	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
106	78	M	DCLD	Y			Blood, Urine	No growth	No growth																							
107	36	M	DCLD	Y			Ascitic fluid,Blood	No growth	Klebsiella pneumoniae (ESBL)		S	S	R	S	R		S	S													
108	35	M	DCLD	Y			Ascitic fluid,Blood	No growth	Stapylococcus aureus (MRSA)		S	S			S					R	S		S	R					S		R	
109	57	M	DCLD	Y			Wound swab,Blood	No growth	Staphylococcus epidermidis(MS)		R	R			S					S	S		R	R					S		S	
110	45	M	DCLD	Y			URINE,Blood	No growth	No growth																							
111	30	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
112	45	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
113	35	M	DCLD	Y			Ascitic fluid,Blood	No growth	E.coli (ESBL)		S	S	R	S	R		S	S													

S. No	Patient's Age	Sex	Diagnosis	Causes of cirrhosis			Sample	CULTURE RESULT		Complememnt C3 level [mg/ml]	Antibiotic sensitivity																					
				Alcohol	Viral	Cryptogeni		Blood	OTHER SAMPLE		Amik	Genta	Cef	CS	Cip	Ofl	Imi	Pip/Taz	Chlor	Tetra	Cot	Clin	Ery	Pen	Ceph	Van	NOR	Nitr	Amp	Amox	Cefoxitin	HLG
114	47	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella oxytoca (ESBL)		R	S	R	S	R		S	S			R										
115	52	M	DCLD	Y	Y		Ascitic fluid,Blood	No growth	No growth																							
116	65	M	DCLD	Y			Ascitic fluid,Blood	No growth	Stapylococcus aureus (MRSA)		S	S			S					R	R		S	R					S		R	
117	54	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth																							
118	45	M	DCLD	Y			URINE,Blood	No growth	E.Coli (ESBL)		S	S	R	S	R		S	S									R	S				
119	51	M	DCLD	Y			Blood, Wound swab	Pseudmonas aeruginosa	No growth		S	S	R	R		R	S	S														
120	41	M	DCLD	Y			Blood, Sputum	No growth	Normal throat commensal																							
121	56	M	DCLD	Y			Blood, Urine	No growth	No growth																							
122	48	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth																							
123	45	M	DCLD	Y			Wound swab,Blood	No growth	Stapylococcus aureus (MRSA)		S	R			R					S	S		S	R					R		R	
124	30	F	DCLD			Y	Ascitic fluid,Blood	No growth	No growth																							
125	50	M	DCLD	Y			Wound swab,Blood	No growth	Klebsiella pneumoniae (ESBL)		S	R	R	S		R	S	S			S										
126	40	M	DCLD	Y			URINE,Blood	No growth	E.Coli		R	R	R	R	R		S	R									S	R				
127	34	M	DCLD		Y		Wound swab,Blood		No growth																							
128	59	M	DCLD	Y			Sputum, Blood	No growth	Pseudomonas aeruginosa		S	S	S	S	S		S	S			S										
129	49	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca (ESBL)		S	R	R	S	S		S	S									R	S				
130	42	M	DCLD	Y			URINE,Blood	No growth	No growth																							
131	38	M	DCLD	Y			URINE,Blood	No growth	Klebsiella oxytoca		S	S	S	S	S		S	S									R	R				
132	39	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
133	60	M	DCLD	Y			Wound swab,Blood	No growth	Proteus vulgaris (ESBL)		S	S	R	S		S	S	R			S										
134	57	M	DCLD	Y			URINE,Blood	No growth	No growth																							
135	60	M	DCLD	Y			Blood, Sputum	No growth	Normal throat commensal																							
136	39	M	DCLD	Y	Y		Ascitic fluid,Blood	No growth	Enterococcus faecalis										S	R			R	S		R			S			S
137	46	M	DCLD	Y			Ascitic fluid,Blood	No growth	E.Coli (ESBL)		R	R	R	S	R		S	S														
138	35	M	DCLD	Y			URINE,Blood	No growth	No growth																							
139	30	M	DCLD	Y			Blood, Wound swab	E.Coli	,No growth		S	R	R	R	R		S	S														
140	49	M	DCLD	Y			Ascitic fluid,Blood	No growth	Klesiella pneumoniae (ESBL)		S	R	R	S	S		S	R														
141	43	M	DCLD	Y			Sputum, Blood	No growth	Klebsiella oxytoca (ESBL)		S	R	R	S	S		S	S			R										
142	52	M	DCLD	Y			Ascitic fluid,Blood	No growth	No growth																							
143	58	M	DCLD	Y			Blood, Urine	No growth	No growth																							
144	38	M	DCLD	Y			Ascitic fluid,Blood	No growth	Enterobacter spp (ESBL)		S	R	R	S	R																	
145	66	M	DCLD	Y			Ascitic fluid,Blood	No growth	E.coli		S	S	S	S	S		S	S			S											
146	56	F	DCLD		Y		Wound swab,Blood	No growth	Stapylococcus aureus (MSSA)		S	S			R					S	S		S	R					S		S	
147	40	M	DCLD	Y			URINE,Blood	No growth	Acinetobacter Spp (ESBL)		S	S	R	S	R		S	S									R	S				
148	60	M	DCLD	Y			Blood, Ascitic fluid	No growth	No growth																							
149	47	M	DCLD	Y			Sputum, Blood	No growth	Normal thoat commensal grown																							
150	58	M	DCLD	Y			URINE,Blood	No growth	No growth																							

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